Proceedings of the
NATIONAL ACADEMY OF SCIENCES

Volume 40 • Number 8 • August 15, 1954

*THE EFFECT OF DIABETES AND FASTING ON LIVER GLUCOSE-6-PHOSPHATASE**

By James Ashmore,† A. Baird Hastings, and Frances B. Nesbett

DEPARTMENT OF BIOLOGICAL CHEMISTRY, HARVARD MEDICAL SCHOOL, BOSTON, MASSACHUSETTS

Communicated July 2, 1954

Studies on the metabolism of C^{14}-labeled fructose, glycerol, and pyruvate by rat liver slices[1] have shown that livers from diabetic rats exhibit an increased production of glucose and a decreased production of glycogen from these substrates, as well as a diminished uptake of glucose. Shipley and Humel,[2] using nonisotopic substrates, have previously observed that liver slices from diabetic rats produce more glucose than slices from normal rat liver. Stetten *et al.*[3] have estimated serum glucose production by isotopic dilution in intact normal and diabetic rats during the constant infusion of C^{14}-labeled glucose. Their data suggest an increased glucose synthesis in the diabetic over the normal. These findings led us to determine whether the activity of glucose-6-phosphatase was altered in diabetic liver tissue.

Drabkin and Marsh[4] have found liver acid and alkaline phosphatases elevated in alloxan-diabetic rats. Cori and Cori[5] have reported that liver glucose-6-phosphatase is drastically lowered in patients suffering from glycogen storage disease. We have determined the activity of this enzyme in liver from fed and fasted normal and diabetic rats. Two methods of assay have been used: (*a*) measuring the release of inorganic phosphate on incubation of G-6-P[6] with liver homogenate, as previously described by Swanson[7] and De Duve *et al.*,[8] and (*b*) measuring the oxygen uptake in a coupled glucose-6-phosphatase, glucose oxidase plus catalase system.

MATERIAL AND METHODS

Albino rats of the Wistar strain, weighing between 200 and 300 gm., were used. Alloxan-diabetic animals were produced by intravenous injection of 40 mg/kg of alloxan monohydrate (Eastman). These animals were not used until at least 15 days after alloxan administration, and only when their blood sugar was greater than 300 mg. per cent. All animals were maintained on water and Purina Laboratory Chow ad lib. To determine the effect of insulin, alloxan-diabetic rats were injected with 5 units of protamine Zn insulin at 12-hour intervals, beginning 48 hours prior to sacrifice of the animals.

Animals were killed either by a blow on the head or by exsanguination under sodium amytal anesthesia. The liver was removed, and 0.50 gm. was homogenized

in 6 ml. of water or isotonic KCl, using a Potter-Elvehjem glass homogenizer. Neither the method of sacrifice nor the solution used for homogenization was found to influence liver phosphatase activity. The homogenate was centrifuged at room temperature for 5 minutes at 1,800 rpm in an International No. 2 centrifuge.

Phosphate Release Assay.—Aliquots of the liver homogenate were assayed according to the method of Swanson.[7] A pH activity curve (Fig. 1) was plotted for the various preparations studied. In this assay our data on optimum pH and relative rates of hydrolysis of other hexose phosphates by liver homogenates are in agreement with those of others.[5, 7, 8] The failure of Be ion at 10^{-3} *M* to inhibit glucose-6-phosphatase activity of our preparations is in accord with similar observations of Cochran *et al.*[9] and constitutes further evidence that this enzyme is quite different from other phosphatases, alkaline phosphatase (serum and tissue), ATP-ase, and phosphoglucomutase, which are strongly inhibited by this ion.

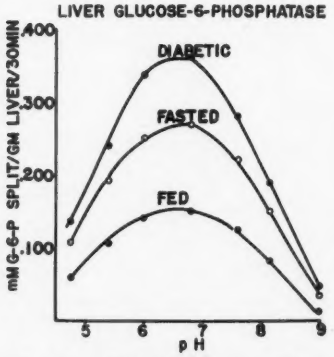


FIG. 1.—pH activity curve of liver glucose-6-phosphatase from fed and fasted normal and diabetic rats. Citrate buffer (0.1 *M*) was used for the pH range 4.76–6.88, and 0.1 *M* borate buffer for pH 7.6–9.0. Each curve represents the mean of six or more animals.

Glucose Release Assay.—This method depends upon the measurement of the glucose released from G-6-P. The glucose liberated was converted to gluconic acid by glucose oxidase in the presence of catalase, and the oxygen consumed was measured manometrically.

One hundred milligrams of glucose oxidase (Sigma) and 0.1 mg. of crystalline catalase (Worthington) were suspended in 5 ml. of 0.154 *M* acetate buffer, pH 5.7. One milliliter of this suspension was mixed with 0.5 ml. of liver homogenate (0.50 gm liver wet weight/6 ml of water) and 0.5 ml. of 0.2 *M* G-6-P, and the oxygen uptake was measured in a Scholander respirometer[10] at 30° C. Appropriate controls on substrate and homogenate, with and without glucose oxidase present, were used.

Keilin and Hartree[11] have reported that glucose oxidase is highly specific for glucose and does not act on any of the closely related carbohydrates, including hexose-6-phosphate. We have confirmed these observations. Incubation of G-6-P as a substrate with liver homogenate and glucose oxidase plus catalase therefore results in oxygen uptake only after liver glucose-6-phosphatase has split G-6-P to glucose and inorganic phosphate. If the phosphatase was the rate-limiting step and all other components were present in excess, the activity of the phosphatase could be measured. From Figure 2 it can be seen (curve *1*) that when glucose was the substrate, the oxygen uptake was three times as great per unit time as with G-6-P (curve *2*). Thus the glucose oxidase–catalase system was present in excess and was not rate-limiting. Likewise, the homogenate without added G-6-P (curve *4*) showed little oxygen consumption, and the substrate (G-6-P) plus glucose oxidase without homogenate (curve *5*) even less. The fact that the oxygen-uptake curves (*2* and *3*) were linear over the time of the assay indicates that the substrate (G-6-P) was present in excess and did not become rate-limiting during the course of the assay. Also, if, as others have shown,[5, 7, 12] glucose is produced by

the liver homogenate only as a result of the action of glucose-6-phosphatase on G-6-P, then the small oxygen uptake in curve *4* (homogenate plus glucose oxidase without added G-6-P) is probably the result of endogenous production of G-6-P by the liver homogenate. This would result in increasing the amount of substrate (G-6-P). which was already present in excess. The oxygen uptake of curve *4* has therefore not been subtracted from the assay determination. Liver homogenates plus G-6-P alone resulted in no consumption of oxygen.

The two assays are compared in Table 1. It can be seen that the glucose-6-phosphatase activity was approximately the same by either assay, the average deviation being ±12 per cent.

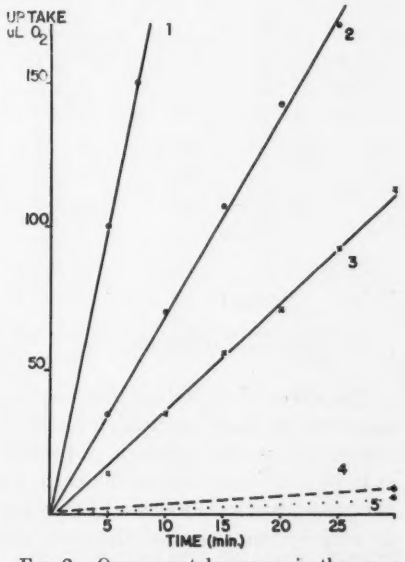


FIG. 2.—Oxygen-uptake curves in the assay for glucose-6-phosphatase. *1*, Glucose control: 0.5 ml. liver homogenate plus 1 ml. glucose oxidase solution in 0.154 *M* acetate buffer (pH 5.7) plus 0.5 ml. 0.2 *M* glucose. *2*, Diabetic liver: 0.5 ml. diabetic liver homogenate plus 1 ml. glucose oxidase solution plus 0.5 ml. 0.2 *M* G-6-P. *3*, Normal liver: same as *2*, except normal liver homogenate was used instead of diabetic. *4*, Control without substrate: 0.5 ml. homogenate plus glucose oxidase. *5*, Control without homogenate: G-6-P plus glucose oxidase.

RESULTS

In Table 2, glucose-6-phosphatase activity in rat livers under the several experimental conditions examined is presented in two ways—activity per gram of wet liver and activity per total liver. The results obtained by both methods of assay show that liver glucose-6-phosphatase was elevated in the fasting and alloxandiabetic rat, when expressed per gram of liver.

Although fasting and alloxan diabetes resulted in an increased concentration of glucose-6-phosphatase, the total quantity of this enzyme present in the liver was elevated only in the fed alloxandiabetic rat. Fasted normal rats had twice the glucose-6-phosphatase activity per gram of liver that fed normal rats had, but, since fasting resulted in a 50 per cent decrease in liver weight, the total phosphatase per liver remained the same. It would therefore appear that fasting in the normal rat resulted in an increased concentration of this enzyme rather than an accelerated synthesis or an activation process. Fasting in the diabetic rat resulted in a net decrease in total liver glucose-6-phosphatase, although the amount of enzyme per gram of liver was unchanged. Fasted normal and alloxan-diabetic rats had the same concentrations of glucose-6-phosphatase per gram of liver, as well as the same total liver glucose-6-phosphatase.

When equal quantities of liver homogenate from fed and fasted normal rats were mixed, the resulting phosphatase activity was the expected arithmetic mean of the component homogenates (Table 3). This again suggests that one was not dealing with a process of activation or inhibition. In the case of the fed diabetic rat, there

was an increase in the total liver glucose-6-phosphatase, as well as in the concentration of the enzyme in the liver (Table 2). Again, a mixture of equal quantities of homogenate from normal and diabetic livers resulted in an activity approaching the mean of the components (Table 3).

TABLE 1

A COMPARISON OF GLUCOSE-6-PHOSPHATASE ACTIVITY IN RAT LIVER HOMOGENATE BY TWO METHODS OF ASSAY*

Rat No.	Phosphate Release	Glucose Release	d
1	0.320	0.345	+0.025
2	.475	.408	− .067
3	.336	.330	− .006
4	.362	.362	± .000
5	.156	.180	+ .024
6	.168	.180	+ .012
7	.188	.258	+ .070
8	.462	.350	− .112
9	.336	.408	+ .072
10	.308	.312	+ .004
11	.368	.337	− .031
12	.424	.365	− .059
13	0.346	0.365	+0.019
Mean†	0.327	0.323	±0.039

* Values expressed as millimoles of G-6-P split per gram of liver per 30 minutes at 30° C.
† Average deviation, 11.9 per cent.

The effect of inhibitors that combine reversibly with enzymes can be partially overcome by dilution,[13] which results in dissociation of the enzyme inhibitor complexes. Thus the presence of natural inhibitors can be detected by measuring enzyme activity at various dilutions,[14] an increase in activity on dilution suggesting the presence of an inhibitor. Applying this criterion, we have observed no increase in activity on two and threefold dilution of this enzyme. These observations suggested that there was, in normal rat liver tissue, no inhibitor present which was removed by fasting or diabetes.

The in vivo injection of insulin returned the glucose-6-phosphatase activity of diabetic rats toward normal (Table 2). This suggested that the enzyme was elevated in diabetic rat liver, due to conditions arising from the lack of insulin. No in vitro action of insulin on rat liver glucose-6-phosphatase was observed.

CONCLUSIONS

A manometric method has been developed for the assay of glucose-6-phosphatase in rat liver homogenates. This method differed from the phosphate-release method previously used[5, 7, 8] in that the glucose released from G-6-P was measured, instead of inorganic phosphate. Glucose was determined by oxygen uptake in the presence of glucose oxidase and catalase. Both methods gave comparable values for glucose-6-phosphatase when applied to the same homogenate.

Rat liver glucose-6-phosphatase, as previously demonstrated by others,[5, 7, 8] has an optimum pH from 6 to 7 and is not inactivated by Be ion at 10^{-3} M. By these criteria, this enzyme is markedly different from nonspecific organic phosphatases of liver.

The concentration of glucose-6-phosphatase in rat liver was elevated by fasting and alloxan diabetes. That the same enzyme was elevated under these conditions

is suggested by the identity of the pH optima shown by the pH activity curves (Fig. 1). The presence of activators or inhibitors that might account for alterations

TABLE 2

RAT LIVER GLUCOSE-6-PHOSPHATASE*

	No. Obs.	Mean	S.E.	P
ANIMALS FED:				
Normal:				
Phosphate release assay:				
mM G-6-P per gm. liver	13	0.147	0.011	...
mM G-6-P per total liver	6	2.37	.140	...
Glucose release assay:				
mM G-6-P per gm. liver	6	0.195	.019	...
Diabetic:				
Phosphate release assay:				
mM G-6-P per gm. liver	11	0.336	.019	<0.01†
mM G-6-P per total liver	6	5.25	.468	< .01†
Glucose release assay:				
mM G-6-P per gm. liver	3	0.358	.005	< .01†
Diabetic and insulin (in vivo):				
Phosphate release assay:				
mM G-6-P per gm. liver	6	0.158	.008	< .01‡
ANIMALS FASTED:				
Normal:				
Phosphate release assay:				
mM G-6-P per gm. liver	13	0.268	.008	< .01†
mM G-6-P per total liver	7	2.60	.142	> .05§
Glucose release assay:				
mM G-6-P per gm. liver	5	0.362	.026	< .01†
Diabetic:				
Phosphate release assay:				
mM G-6-P per gm. liver	7	0.288	.026	> .05‡‖
mM G-6-P per total liver	4	3.00	.196	> .05#
Glucose release assay:				
mM G-6-P per gm. liver	6	0.362	0.016	>0.05‡‖

* Values expressed as millimoles of G-6-P split per 30 minutes. Calculations per gram of liver (wet weight) and per total liver weight. Phosphate release assays were incubated in 0.1 *M* citrate buffer (pH 6.2) and the glucose release assays in 0.154 *M* acetate buffer (pH 5.7). All assays were at 30° C.
† Calculated *P*-value as compared with normal fed animals.
‡ Calculated *P*-value as compared with diabetic fed animals.
§ Difference not statistically significant as compared with normal fed animals.
‖ Difference not statistically significant as compared with diabetic fed animals.
Difference not statistically significant as compared with normal fasted animals.

TABLE 3

GLUCOSE-6-PHOSPHATASE ACTIVITY RESULTING FROM MIXTURES OF HOMOGENATES OF LIVER FROM FED AND FASTING NORMAL AND DIABETIC RATS*

FED NORMAL AND DIABETIC RATS (MIXED 1:1)		FED AND FASTED NORMAL RATS (MIXED 1:1)	
Obs.	Calc.	Obs.	Calc.
0.350	0.342	0.212	0.209
.356	.313	.243	.246
.284	.268	.265	.228
.272	.268	.281	.265
0.237	0.212	.243	.246
		0.236	0.215
Mean 0.300	0.281	Mean 0.247	0.235

* Values expressed as millimoles of G-6-P split per gram of liver per 30 minutes. Incubations were carried out at 30° C. in 0.1 *M* citrate buffer, pH 6.2.

in enzyme activity was not detected. It is therefore suggested that the increased activity of this enzyme in fasting and diabetes was due to an actual increase in concentration of enzyme.

Although fasting increased the concentration of glucose-6-phosphatase in the normal rat liver, it caused a net decrease in the total glucose-6-phosphatase in the diabetic liver but did not alter the concentration of the enzyme in the diabetic. The similarity of the glucose-6-phosphatase contents of livers from fasted normal and diabetic rats was striking. Indeed, in so far as this enzyme was concerned, fasting tended to make the normal and the diabetic states indistinguishable.

The increased concentration of glucose-6-phosphatase in diabetic liver could be returned toward normal by the in vivo injection of insulin. In vitro insulin was without effect. This suggests that liver glucose-6-phosphatase was elevated in the diabetic liver due to general metabolic changes that occur in diabetes.

The nature of the changes in glucose-6-phosphatase that occur in diabetes suggests that this enzyme adapts in response to a decreased ability of the peripheral tissues to utilize glucose, thus bringing about an increase in the concentration of blood sugar. In fasting, glucose-6-phosphatase acts to release glucose arising from both glycogenolytic and gluconeogenic processes. In diabetes the increase in this enzyme offers evidence for the overproduction of glucose believed to occur in this disease.

* This work was supported in part by the United States Atomic Energy Commission and the Eugene Higgins Trust, through Harvard University.

† Schering Fellow of the Endocrine Society for 1953–1954.

[1] A. E. Renold, Ching-Tseng Teng, F. B. Nesbett, and A. B. Hastings, *J. Biol. Chem.*, **204**, 533, 1953; J. Ashmore, A. E. Renold, F. B. Nesbett, and A. B. Hastings, *Federation Proc.*, **13**, No. 1, 176, 1954.

[2] R. A. Shipley and E. J. Humel, Jr., *Am. J. Physiol.*, **144**, 51, 1945.

[3] De Witt Stetten, Jr., I. D. Welt, D. J. Ingle, and E. H. Morley, *J. Biol. Chem.*, **192**, 817, 1952.

[4] D. L. Drabkin and J. B. Marsh, *J. Biol. Chem.*, **168**, 777, 1947; **171**, 455, 1947; D. L. Drabkin, *Proc. Am. Diabetes Assoc.*, **8**, 3, 1948.

[5] G. T. Cori and C. F. Cori, *J. Biol. Chem.*, **199**, 661, 1952.

[6] As used in this manuscript, "G-6-P" refers to glucose-6-phosphate disodium salt.

[7] M. A. Swanson, *J. Biol. Chem.*, **184**, 647, 1950.

[8] C. de Duve, J. Berthet, H. G. Hers, and L. Dupret, *Bull. Soc. Chim. biol.*, **31**, 1242, 1949.

[9] K. W. Cochran, M. M. Zerwick, and K. P. Du Bois, *J. Pharmacol. Exptl. Therap.*, **102**, 165, 1951.

[10] P. F. Scholander, C. L. Claff, J. R. Andrews, and D. E. Wallach, *J. Gen. Physiol.*, **35**, 375 1952.

[11] D. Keilin and E. F. Hartree, *Biochem. J.*, **42**, 221, 1948.

[12] P. Fantile and M. N. Rome, *Australian J. Exptl. Biol. Med. Sci.*, **23**, 21, 1945.

[13] R. G. Hussey and J. H. Northrop, *J. Gen. Physiol.*, **5**, 335, 1922–1923; A. Goldstein, *J. Gen. Physiol.*, **27**, 529, 1943.

[14] F. T. Brayer and J. B. Trunnel, *Texas Repts. Biol. and Med.*, **10**, 946, 1952.

*COLLAGEN STRUCTURES CONSIDERED AS STATES OF AGGREGATION OF A KINETIC UNIT. THE TROPOCOLLAGEN PARTICLE**

By Jerome Gross, John H. Highberger, and Francis O. Schmitt

DEPARTMENT OF MEDICINE, MASSACHUSETTS GENERAL HOSPITAL,† AND HARVARD MEDICAL SCHOOL, BOSTON; UNITED SHOE MACHINERY CORPORATION, BEVERLY, MASSACHUSETTS; AND DEPARTMENT OF BIOLOGY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE, MASSACHUSETTS

Communicated June 28, 1954

Collagen reconstituted from acid solutions may assume not only the fibrous cross-striated form, having an axial repeating period averaging about 640 A, but also the fibrous long-spacing (FLS) form, having an axial repeating period ranging between 1,500 and 3,000 A and averaging about 2,000 A.[1, 2] This suggested the existence of extremely thin collagenous particles having lengths equal to those of the long spacings; it was supposed that the FLS might be formed by end-to-end and lateral aggregation of these particles. When this concept was formulated, we adopted the term "tropocollagen"[3] to denote the thin, long particles. It was then discovered[4] that a segmental form of collagen long spacing (SLS) may be produced from mildly alkaline salt extracts of collagen by dialysis against citrate buffer or by addition of adenosine triphosphoric acid (ATP) to an acid solution of collagen. This was presumptive morphological evidence in support of the tropocollagen hypothesis. The segments have the lengths ascribed to the thin particles, and it was postulated that in this case the tropocollagen particles line up laterally but not linearly. The fact that collagen, FLS, and SLS may be converted into each other is further support of the view that the three forms represent different states of aggregation of a unit particle (tropocollagen). It was also considered possible that, in the biogenesis of collagen, tropocollagen may be the form synthesized by the cells and passed by them into the ground substance, where it is converted into the characteristic fibrous form.

Evidence for the existence of tropocollagen is primarily morphological (electron microscopic) and is derived from studies of the structures precipitated from collagen solutions. We have also applied physical-chemical methods designed to give information concerning the size and shape of the hypothesized particles in solution. These studies have contributed relatively little interpretable data thus far, probably because the particles exist in solution as thin, extremely long aggregates. If the solution contained a large proportion of very long fibrous polymers of tropocollagen, it would be extremely difficult to demonstrate the presence of a small amount of the monomer.

In the present paper is summarized the evidence thus far obtained that the various morphologically distinguishable forms of collagen are mutually interconvertible and that the unit of collagen structure involved in these changes of aggregation is the tropocollagen particle.

INTERCONVERTIBILITY OF COLLAGEN, FIBROUS LONG SPACING, AND SEGMENT LONG SPACING

This section provides the type of data on which was based the statement[4] that collagen, FLS, and SLS are reversibly interconvertible. Two types of experiments were performed: (1) the production of collagen fibrils, FLS, and SLS, each in pure

form, from different aliquots of the same starting material; (2) quantitative conversion of one form into another. The starting material for these experiments was an acetic acid solution of ichthyocol obtained in the manner previously described.[5] This solution is clear, highly viscous, and contains 0.1–0.2 per cent protein. No structured fibrils are observed but only very thin filaments, the widths of which are near to and probably below the resolution of the electron microscope. Light-scat-

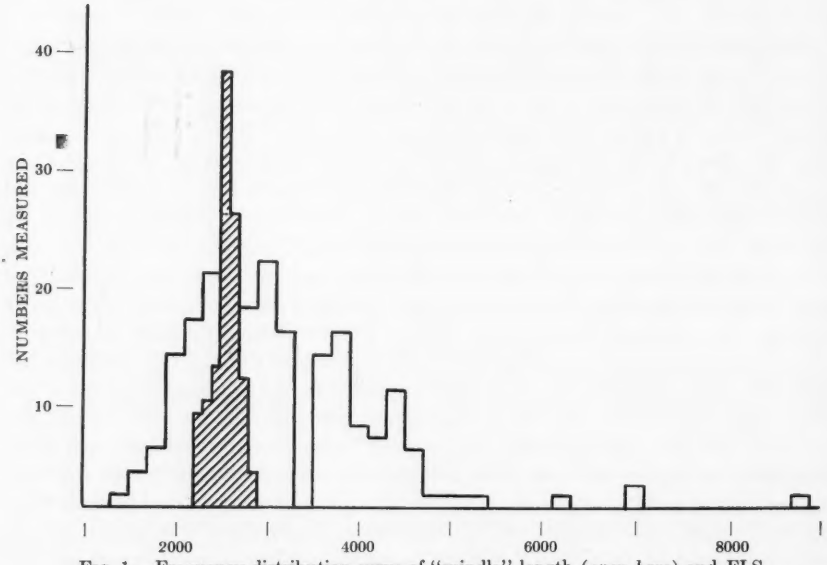


FIG. 1.—Frequency-distribution curve of "spindle" length (*open bars*) and FLS periodicity (*blocked bars*).

tering data[6, 7] on other acid-dissolved collagen solutions suggest that the weight of the dissolved particles is of the order of 10^7. It is possible that the bulk of the collagen is in the form of highly solvated single protofibrils[8] of varying lengths.

Aliquots of ichthyocol acid filtrate were treated as follows:

a) 100 ml. were dialyzed against two changes of 2 liters each of 0.17 *M* NaCl solution at room temperature for 24 hours. The resulting precipitate consisted of collagen fibrils in which detailed fine structure was observed; sometimes unstructured filaments were seen, but these represented a small fraction of the total. As much as 80 per cent of the original dissolved collagen was precipitated in fibrillar form.

b) To 50 ml. was added an equal volume of a 0.02 per cent solution of human serum α 1 acid glycoprotein[9] in 0.05 per cent acetic acid. The mixture was dialyzed overnight against cold running tap water. The heavy white flocculent precipitate

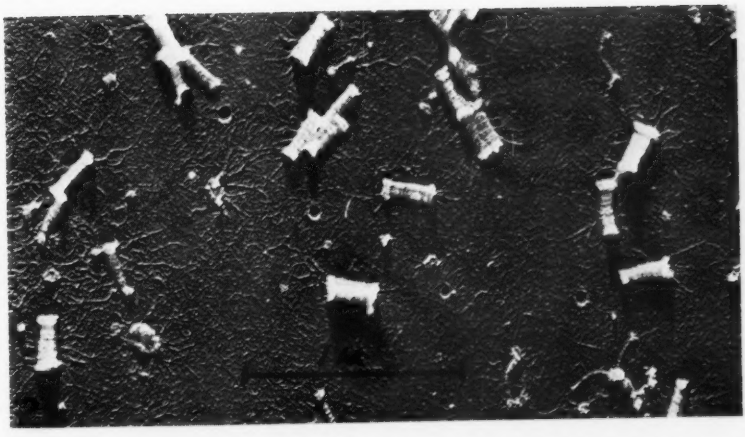
FIG. 2.—Frayed segment long spacing (SLS), demonstrating thin longitudinal components of segments. Chromium-shadowed. ×33,000.

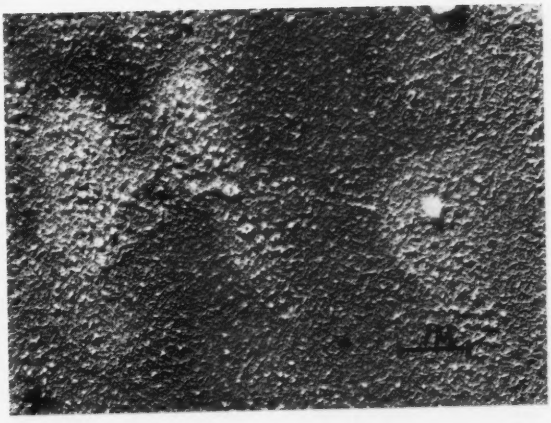
FIG. 3.—Mixture of ichthyocol solution and glycoprotein before dialysis. Chromium-shadowed. ×10,000.

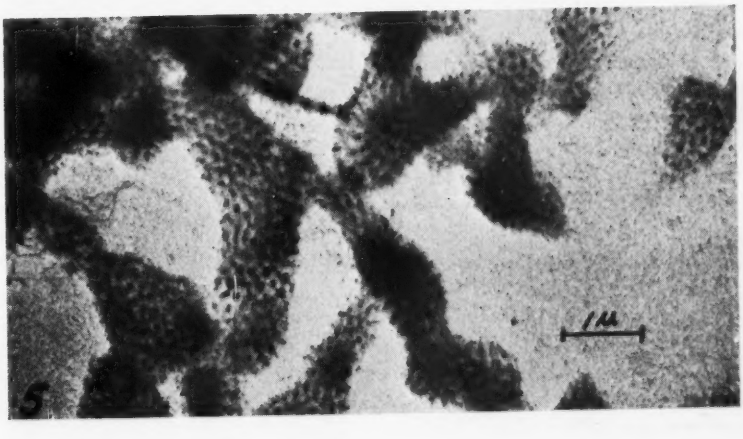
FIG. 4.—Same mixture as in Fig. 3 after dialyzing against water for 15 minutes. Earliest appearance of "spheroids." Chromium-shadowed. ×10,000.

FIG. 5.—Same preparation after dialyzing 45 minutes. Phosphotungstic acid-stained. ×11,700.

PLATE I







was composed entirely of FLS, as nearly as could be judged by examination in the electron miscroscope. The precipitated FLS accounted for 60–80 per cent of the original dissolved collagen.

c) To 100 ml. was added an equal volume of 0.2 per cent ATP in 0.05 per cent acetic acid, and the mixture was allowed to stand for 18 hours. The precipitate was found to consist almost entirely of SLS; very little filamentous or amorphous material was found. Eighty per cent of the collagen appeared in the SLS precipitate.

In each of the three cases described above the precipitates were spun down at 80,000 g., and the sediments and supernates were lyophilized and analyzed for glycine and hydroxyproline.[10] The supernates usually contained only traces or collagen. Accordingly, one must conclude that the acid-dissolved filmaments of protofibrils were converted nearly quantitatively into collagen fibrils, FLS, and SLS, respectively, in aliquots a, b, and c.

In the second type of experiment FLS and SLS were converted into collagen as follows:

a) Quantitative conversion of FLS to collagen. The transformation from ichthyocol acid filtrate to FLS was performed as described in connection with aliquot b above. The suspension of pure FLS was centrifuged and the sediment resuspended in distilled water, recentrifuged, and lyophilized. Hydroxyproline and glycine were determined. Weighed samples were completely dissolved in 0.05 per cent acetic acid to give approximately 0.1 per cent solutions. The viscous solutions were dialyzed against 1 per cent NaCl at room temperature. The salt solutions (pH $\geqq$ 6) were changed twice. Heavy flocculent precipitates formed which proved to be composed exclusively of collagen fibrils with detailed intraperiod fine structure. The precipitate was sedimented in the Spinco preparative centrifuge; the supernate was dialyzed free of salt, lyophilized, weighed, and analyzed for hydroxyproline and glycine. The precipitate was resuspended in salt solution, sedimented, dialyzed free of salt, lyophilized, weighed, and analyzed. It was found that as much as 80 per cent of the hydroxyproline and glycine in the FLS were recovered in the collagen formed.

b) Quantitative conversion of SLS to collagen. To two aliquots[11] of ichthyocol collagen dissolved in acetic acid were added equal volumes containing identical amounts of the same sample of ATP (free acid dissolved in water). The mixtures were allowed to stand at room temperature overnight. The heavily opalescent solutions were centrifuged at 10,000 g. for 30 minutes, the supernates poured off, and the sediments resuspended in 0.05 per cent acetic acid. The suspensions examined in the electron microscope revealed large masses of typical SLS in clumps, in chains, and as individual segments. There was little filamentous background. Both solutions were recentrifuged, supernates were poured off, and one sediment was transferred quantitatively to a weighed lyophilizing vial, while the other was dissolved in cold 0.15 *M* phosphate buffer, pH = 8. The material dissolved completely after several hours and was extremely viscous. The phosphate solution of SLS was warmed at 32° C. under toluene for 4 hours, forming an opaque white gel. This was broken with a spatula and sedimented by centrifugation. The supernate was dialyzed against water and lyophilized. The fibrous sediment was redispersed in buffer, and specimens were prepared for electron microscopy. As will be described elsewhere, such precipitates are composed of typical cross-striated collagen fibrils

and of no other obvious structures. This material was again sedimented, dialyzed free of salt, lyophilized, and weighed.

The percentage transformation of SLS to collagen closely approximated that obtained with FLS preparations (60–80 per cent).

Unfortunately, employment of processes such as dialysis and lyophilization prevent anything approaching 100 per cent recoveries; material is unavoidably lost on the lyophilizing baffle or on the dialysis bag. Control experiments showed that recoveries seldom exceeded 75 per cent. Similar difficulties have been encountered by others.[12] Since interconversions of one form of collagen to another occurred to a measured extent of 50–80 per cent, it may be concluded that the actual interconversion was significantly higher. The fact that relatively little collagen was found in the supernates after conversion to collagen further supports this view.

It is possible that small fragments of the fibrous protein may be split off in one or another of these transformations (such as occurs, for example, in the splitting-off of fibrinopeptide when fibrinogen clots under normal conditions). We have not as yet examined this possibility experimentally. However, if it does occur with changes of state of tropocollagen, the amount of material split off must be relatively small.

ELECTRON MICROSCOPE OBSERVATIONS BEARING ON STRUCTURE AND PROPERTIES OF TROPOCOLLAGEN

We visualize the tropocollagen particle as being very thin (less than 50A) and of the order of 2,000 A in length. It has not yet been possible to observe these particles directly in the electron microscope. However, certain observations of long-spacing material made in the process either of distintegration or of formation support our general tropocollagen hypothesis. These will now be briefly described.

1. SLS DISINTEGRATING IN ACID

It was previously pointed out[4] that, in the absence of ATP, acid causes the SLS slowly to disintegrate, and such shadowed preparations showed thin particles which appeared to have been liberated from the SLS. Such a preparation is shown in Plate I, Figure 2. Within our ability to measure, the frayed elongate particles have the same length as the segments from which they were derived. The particles have thickness down to the limit of resolution of the preparations, e.g., of the order of 50 A. It seems probable that they represent thin bundles of tropocollagen particles.

2. ELECTRON MICROSCOPE STUDIES OF THE PROCESS OF FORMATION OF LONG-SPACING STRUCTURES

We have followed the process of formation of FLS and SLS in time-sequence studies, and in both cases structures were observed which might well represent thin bundles of tropocollagen particles.

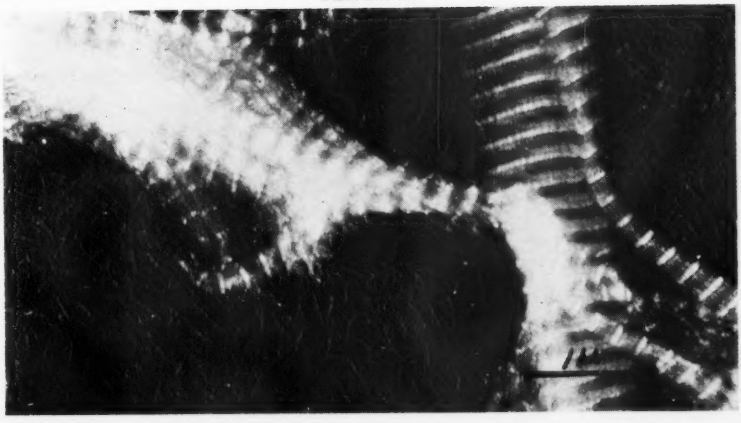
a) Fibrous Long Spacings.—To portions of ichthyocol acid filtrate was added human serum α-1 acid glycoprotein in amounts sufficient to make a final concentra-

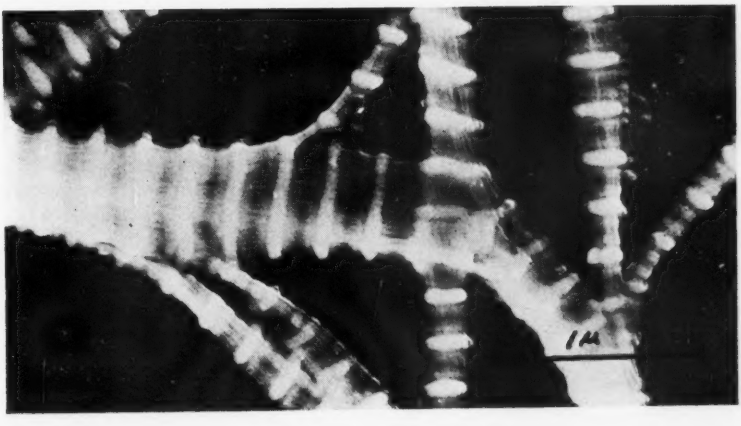
FIG. 6.—Same preparation after dialyzing 75 minutes. Formation of long-spacing fibrils is further advanced, and "spindles" are seen in background. Chromium-shadowed. ×16,000.

FIG. 7.—Same preparation as in Fig. 6, showing another field, permitting a comparison of "spindle" size with the periods of newly formed fibrous long spacing. Chromium-shadowed. ×16,000.

FIG. 8.—Same preparation after 4 hours of dialysis, revealing fully formed SLS and longitudinal intraperiod fibrillations which may represent incorporated "spindles." Chromium-shadowed. ×31,300.

PLATE II





tion of 0.02–0.06 per cent. A sample of this mixture was taken for electron microscope examination (Pl. I, Fig. 3). The mixture was then dialyzed against distilled water at room temperature, and samples were removed at intervals of about 15 minutes, stained with phosphotungstic acid or shadowed with chromium, and examined with the electron microscope.

Two variants of a common pattern of FLS formation were noted which seemed to depend on the particular collagen solution and procedures used in obtaining and processing the sample for electron microscopy.

In one series of experiments the first structures to be observed after dialysis began were dense spheroidal particles 400–800 A in diameter. The distribution curve was fairly symmetrical, the average value being about 550 A. These were not present in the ichthyocol or acid glycoprotein solutions or in the mixture of the two (Pl. I, Fig. 3) but appeared shortly after dialysis began (Pl. I, Fig. 4). We suspect that they were composed of tropocollagen particles which had become anchored to glycoprotein molecules through the negative charges which abound on the latter. Attemps to demonstrate this by isolation of the spheroids and determination of their collagen and glycoprotein content are in progress.

Soon after dialysis began, large cloudlike gelled aggregates formed, in which, sometimes at regularly (hexagonally) spaced intervals, were seen the dense spheroids (Pl. I, Fig. 5). The gel was presumably composed of tropocollagen particles anchored to the glycoprotein-rich centers and interconnected in a three-dimensional net. From the gel emerged FLS fibrils presumably resulting from the anisodiametric ordering of the particles. As the fibers appeared, the "clouds" disappeared. Intermediate stages of condensation were also observed (Pl. II, Fig. 6).

At about the time the cloudlike aggregates condensed to form fibrils, large numbers of fusiform objects became apparent in the cleared background (Pl. II, Figs. 6 and 7), only to disappear again in about 30 minutes as dialysis proceeded. We have referred to these tactoidal objects as "spindles." It is presumed that they form when conditions are suitable; probably concentration, pH, and other factors are critical.

The spindles are about 100–250 A wide at their broadest point and somewhat longer on the average than the periods of the FLS being formed in their midst. In Figure 1 is shown a distribution curve of the FLS periods and the lengths of the spindles, both obtained from the same electron micrographs. The accuracy of measurement of the spindle lengths is rather low. It is probable that the relatively long spindles (7,000–8,000 A) represent tactoidal aggregates several times the length of a tropocollagen particle. The FLS period falls near the middle of distribution of spindle lengths. It seems reasonable to assume that the spindles are composed of a small number of tropocollagen particles in tactoidal aggregation and that the FLS is formed by linear and lateral aggregation of tropocollagen particles, presumably by dovetailing of their ends. Actually, one can see individual filaments (which may be thin bundles of tropocollagen particles) in favorable electron micrographs of FLS (see Pl. II, Fig. 8). The major repeating bands of the FLS may be thick relative to the interband region because of the overlapping of the tropocollagen and because of the presence of dense spheroidal material which is presumably composed of tropocollagen and acid glycoprotein. By analysis it was shown that FLS produced in this manner contained 10–15 per cent glycoprotein.

That the dense spheroids (and perhaps the spindles) are directly involved in the formation of FLS is indicated by the fact that, when the FLS are fully formed, the spheroids and spindles are no longer seen.

In another group of time-sequence experiments using different ichthyocol solutions, the first stage of spheroid formation occurred as described; however, instead of fibrils forming by condensation of cloudlike networks, the next stage involved the formation of separate thin, long periods with what appeared to be spheroids at each end (Pl. III, Fig. 9). Fifteen minutes later the preparation showed, in addition to the separate periods, short linear aggregates ranging upward from two periods and also well-formed short tactoidal and fibrous FLS (Pl. III, Figs. 10 and 11). These latter probably represented either linear and lateral aggregates of single periods or perhaps formation from a single segment nucleus by ordered accretion of tropocollagen particles and glycoprotein.

b) Segment Long Spacings.—Time-sequence studies of the mode of formation of SLS were attempted, as in the above-described studies of FLS. However, this could be done only if the concentration of ATP was properly adjusted. With concentrations of ATP of 0.1 per cent or higher, the precipitation of SLS was usually so rapid that intermediate stages were difficult to observe. Within a matter of 10 seconds or less only fully formed SLS could be found. Occasionally a favorable specimen showed extremely thin SLS attached to fully formed segments (Pl. IV, Fig. 14). Some of the thinner segments must be bundles of only a few tropocollagen particles.

With 0.075 per cent of ATP, precipitation occurred less rapidly. Immediately after mixing equal volumes of a 0.15 per cent aqueous solution of ATP (the free acid) and an acetic acid solution of ichthyocol, a drop of the solution was applied to a grid for less than a second, and the preparation was stained with phosphotungstic acid or shadowed with chromium. Electron microscope examination revealed large numbers of spheroids similar to those observed in the early stages of FLS formation; a small number of fully formed SLS and some very thin SLS with spheroids at their ends were also seen (Pl. IV, Fig. 13). As time passed, the solution gradually increased in opalescence, and simultaneously the number of SLS observable markedly increased, while the number of spheroids decreased. If the concentration of ATP was lower (0.05 per cent), the result was the formation of a large number of spheroids but almost no SLS (Pl. IV, Fig. 12). Standing for 4 hours changes the picture very little.

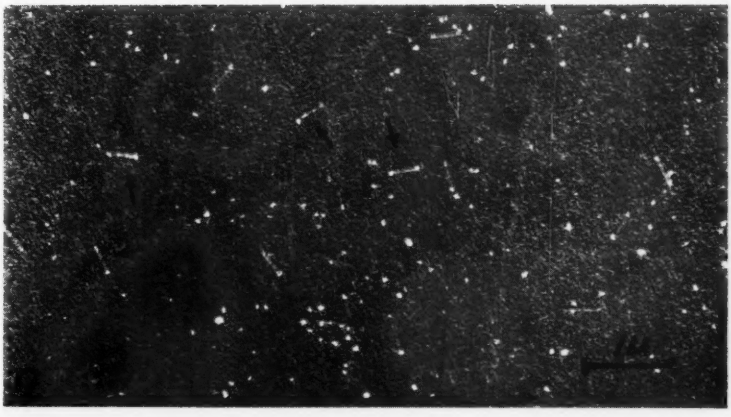
The distribution curve of the spheroid diameters produced with ATP closely resembled that described above for glycoprotein-induced spheroids in the formation of FLS. The mean diameter was about 550 A. Apparently a certain threshold of concentration of ATP is needed before the kinetic requirements of the reaction (i.e., the collision of tropocollagen particles in a manner such that matching groups

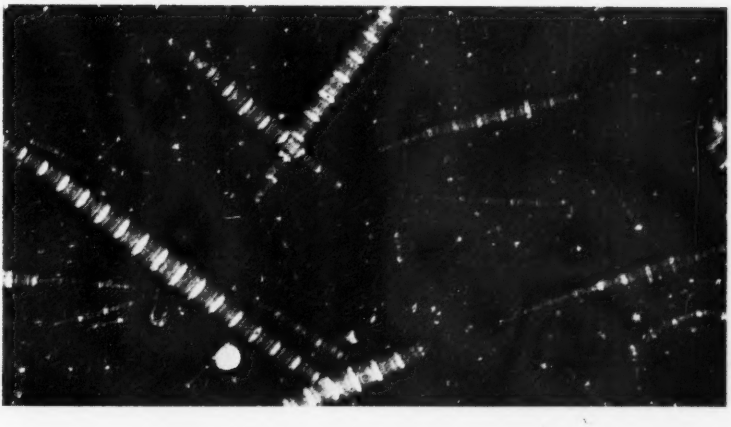
FIG. 9.—Very early stage in formation of FLS, in which individual spheroids and separate thin periods (*arrows*) appear, rather than condensing "clouds," as in Figs. 4 and 5.

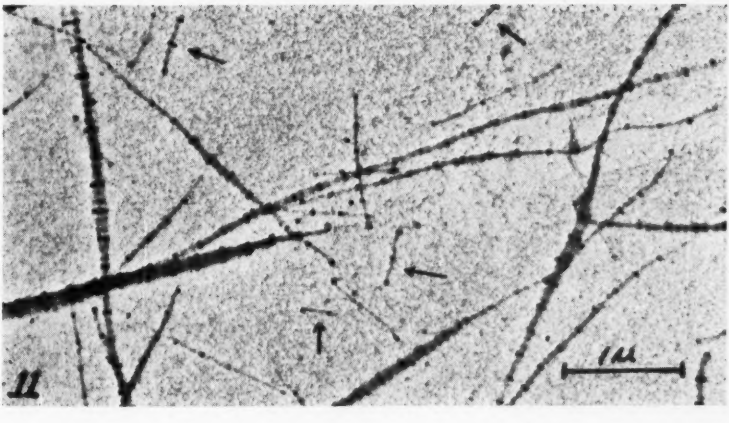
FIG. 10.—Same preparation as in Fig. 9, but later stage in development of FLS, in which are seen individual periods as well as FLS showing different degrees of development. Chromium-shadowed. ×15,700.

FIG. 11.—Same stages in development of FLS as in Fig. 10. Phosphotungstic acid-stained. ×17,000.

PLATE III







are able to exert attraction upon each other) are met. The spheroid stage seems to be a necessary intermediate, presumably because this stage provides nuclei of high concentrations of tropocollagen chains.

The details of the mechanism by which the long threads of collagen protofibrils, in acid solution, undergo scission or depolymerization into tropocollagen under the influence of ATP are not clear. Correspondence between the number of equivalents of lysine and ATP, observed in two SLS preparations, suggests that binding of ATP (and possibly scission of collagen chains) involves this basic residue.

It may be worthy of note that when SLS are formed, at whatever concentrations of ATP and acid solution of collagen, the fine structure is the same.

DISCUSSION

Some of the major problems posed by these experiments are: the physical and chemical nature of the tropocollagen particle; the mechanism whereby tropocollagen may be transformed into collagen (and other reconstituted forms); the physical basis of the band patterns specific for each type of structure, the relationship of tropocollagen to the molecule of collagen; and the role of tropocollagen in the biogenesis (and pathology) of connective tissue.

Difficulties encountered in the physical-chemical characterization of tropocollagen have been alluded to above. Thus far, attempts to obtain solutions of collagen or of material capable of being reconstituted into collagen, which diffuse normally in the ultracentrifuge (i.e., without the formation of hypersharp peaks at low concentrations) have proved fruitless. Treatment with urea (8 *M*) leads to fragments which fulfil the desired physical-chemical criteria. However, even after removal of the urea, we have thus far been unable to reconstitute collagen, FLS, or SLS in significant amounts from such solutions. By heating "procollagen" solutions under controlled conditions, Gallop[7] obtained a homogeneous solution of "parent gelatin" with a molecular weight of about 70,000, but, since this material is a gelatin, it could not be reconstituted into collagen. We are continuing our efforts to produce tropocollagen in a form which is suceptible of rigorous physical-chemical analysis and which is convertible into collagen but in which the great tendency for linear and lateral aggregation is controllable chemically.

Plausible mechanisms are suggested above whereby tropocollagen is converted into FLS and SLS. Similar studies of the mechanism of the transformation of tropocollagen into collagen by dialysis against salt solutions are now being made and will be reported elsewhere. It may be recalled that Porter and Vanamee[13] studied the effect of ionic strength, pH, and time on the reconstitution of collagen from acetic acid filtrates of rat-tail tendon by dipping thin films of the filtrate into the different salt and buffer solutions for various time intervals. In addition to cross-striated and nonstriated fibrils, they observed in the background of several of their preparations numbers of short tactoidal particles, which they stated were "small multiples of 640 A in length," inferring that "at least in acetic acid solutions the basic unit is a very slender molecule 640 A long." No data supporting these statements were offered. Randall[14] has suggested that collagen fibrils may be composed of globular units about 640 A in diameter. The spheroids described herein have diameters of this order of size. However, no evidence has as yet been found that these actually form part of the collagen fibrils (though they may well be involved in the formation of the fibrils).

The fact that the distribution curves of the FLS periods and of the SLS lengths are very broad, ranging from about 1,500 to 3,000 A, might seem peculiar if tropocollagen is a physical entity presumably having a constant or at least definable length. Actually a similar situation exists in the case of collagen, which has an unique period of 640 A, as shown by x-ray diffraction,[15] but which shows axial periods ranging from about 400 to 1,000 A in electron microscope preparations.[16]

In order to compare the variability of the period in FLS and collagen, we have made a preparation in which both fibrous types were produced together. To an acid solution of ichthyocol was added an amount of acid glycoprotein sufficient to produce, after dialysis a mixture of FLS and collagen-type fibrils, as previously described by us.[2] The range of variability of the collagen periods (350–850 A; average, 650 A) was greater on the percentile basis than that found for the FLS periods (1,500–3,100 A; average, 2,400 A).

Schmitt, Hall, and Jakus[16] supposed that the large range of periods seen in electron microscope preparations was due to mechanical distortions inflicted upon the fibrils by the preparative procedures (the largest variations were seen in preparations fragmented with the Blendor). The observations[17] that the relative positions of the collagen intraperiod bands were constant over a large range of periods suggested that the collagen protofibrils might be susceptible of long-range extensibility.

It is possible that similar considerations apply to FLS and SLS, though it is equally possible that other factors not yet identified may play a crucial role in this phenomenon. It may be mentioned in this connection that both FLS and SLS give the same large-angle x-ray pattern as that characteristic of collagen. Also, a few instances have been noted in which FLS fibrils underwent large extensions under the action of the electron beam. It seems clear that the long-spacing forms are not composed of fully extended protofibrils (although some of the intraperiod regions may be highly extended).

The pattern of bands seen in SLS defines this form of collagen at least as well as the band pattern characteristic of fibrous collagen defines that form. For reasons already given, we consider that SLS are formed by the lateral aggregation of tropocollagen particles. Since the band pattern is not symmetrical, we assume that the tropocollagen particles are polarized, i.e. all pointing in the same direction.

The band pattern of fibrous collagen is also polarized, and we have assumed that this is the result of the lateral interaction of neighboring protofibrils all pointing in the same direction.

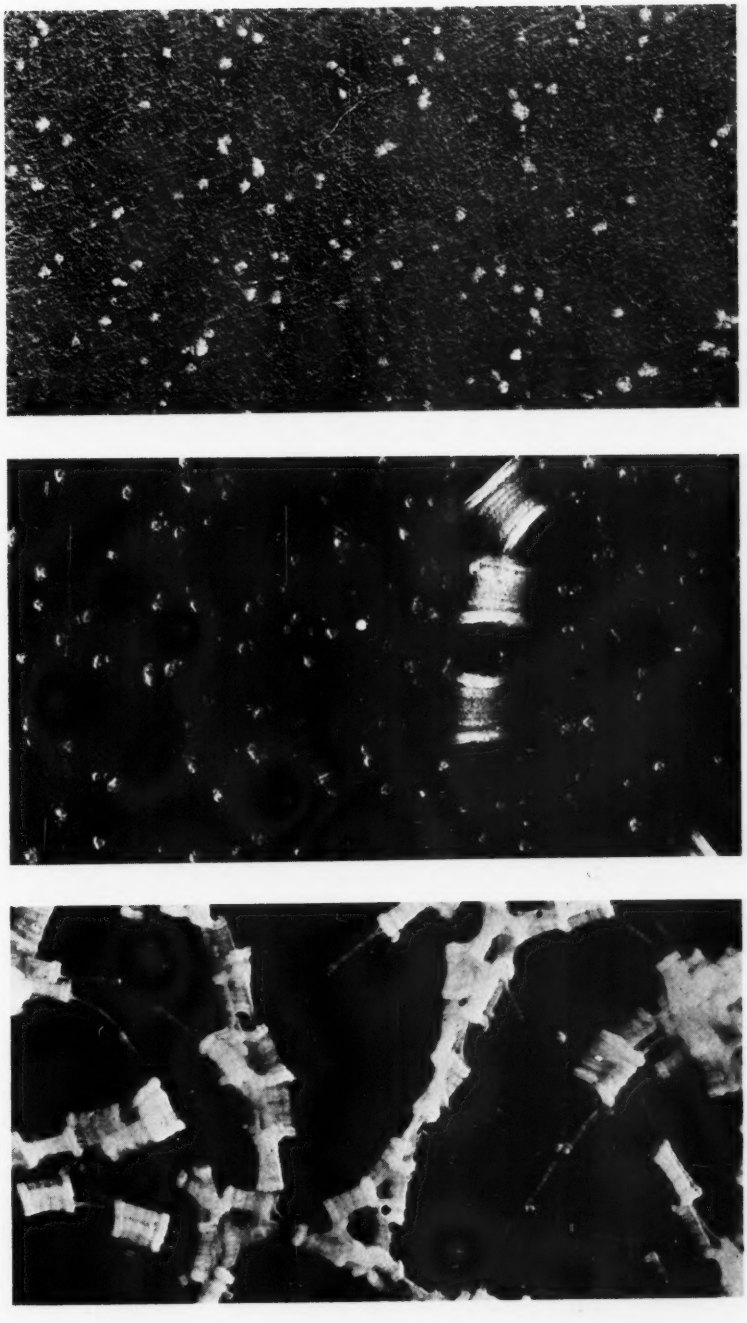
Since the SLS may be taken apart and the component tropocollagen particles reassembled as fibrous collagen with typical band pattern (presumably with no change in the specific pattern of distribution of amino acid residues or residue types), we have entertained the view that the difference between the band patterns of SLS and of collagen is due to a different "phase" relation between matching amino

FIG. 12.—Formation of spheroids in mixtures of ichthyocol solution and 0.05 per cent ATP. No SLS are formed at this concentration. Chromium-shadowed. ×22,000.

FIG. 13.—Formation of spheroids and SLS in ichthyocol solution containing 0.075 per cent ATP. ×37,000.

FIG. 14.—Appearance of SLS in ichthyocol solutions containing 0.1 per cent ATP. Few spheroids are observed. Extremely thin segments appear to be forming on the ends of "mature" SLS. ×33,000.

PLATE IV



acid residue types in adjacent chains. If there is truth in this notion, the tropocollagen particle with a length of the order of 2,000 A would be the minimal particle size truly characteristic of collagen, rather than the 640 A unit.

One final point may be mentioned in connection with the time-sequence studies of the production of FLS and SLS. Although a mechanism was suggested, based on the formation of nuclei of tropocollagen chains co-ordinated about the negative charges of the acid glycoprotein or the ATP, we do not wish to imply that these are unique mechanisms. As previously suggested,[5] the specificity of structure resides in the collagen (or perhaps, more correctly, in the tropocollagen) rather than in the evocating agents. However, quite possibly, substances such as glycoprotein, ATP, and various other agents determine the type of structure which will be reconstituted by the manner in which they combined with the tropocollagen particles, favoring either parallel (SLS) or antiparallel (FLS) packing.

It is worth noting that we have never been able to make SLS and FLS from pure collagen solutions without the addition of some evocating agent. All connective-tissue extracts from which SLS or FLS have been formed without adding other materials have been shown to contain significant amounts of noncollagenous substances. However, typical collagen fibrils are readily precipitated in large quantity from the purest collagen solutions we have been able to prepare.

SUMMARY

Evidence is presented in support of the view that the various structural forms of collagen, including the fibrous and segmental long-spacing type, may be composed of very thin, possibly protofibrillar, particles having lengths in the long-spacing range (1,500–3,000 A, but primarily in the range of 1,800–2,300 A). The hypothesized particles are called "tropocollagen."

Evidence for the existence of tropocollagen is as follows:

1. From a common starting solution of ichthyocol dissolved in acid, collagen, fibrous long spacing, or segment long spacing may be precipitated in separate aliquots almost quantitatively.

2. Each of these forms may be redissolved and converted almost quantitively into either of the other forms.

3. Frayed segments of SLS reveal that they are composed of extremely thin filaments (possibly a few tropocollagen particles thick) having lengths equal to those of the SLS.

4. Time-sequence studies of the formation of FLS and SLS reveal that very thin tactoidal particles of the order of 2500 A in length appear for a short time and then disappear as the long-spacing structure is formed. FLS may also be produced by adlineation of individual separate periods.

Dense spheroids of the order of 500 A in diameter formed early in the precipitation of FLS and SLS and were considered to consist of clusters of tropocollagen particles co-ordinated around the negative groups of ATP or acid glycoprotein.

* This investigation was supported in part by a grant #A90 (C4) from the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service, to the Massachusetts General Hospital and from the Eli Lilly Company to the Massachusetts Institute of Technology.

† This is Publication No. 163 of the Robert W. Lovett Memorial Foundation for the Study of Crippling Disease, Harvard Medical School, and Department of Medicine, Massachusetts General Hospital.

[1] J. H. Highberger, J. Gross, and F. O. Schmitt, *J. Am. Chem. Soc.*, **72**, 3321, 1950.

[2] J. H. Highberger, J. Gross, and F. O. Schmitt, these PROCEEDINGS, **37**, 286, 1951.

[3] From the Greek *tropos*, meaning "to turn" or "to change," hence a substance capable of turning into collagen.

[4] F. O. Schmitt, J. Gross, and J. H. Highberger, these PROCEEDINGS, **39**, 459, 1953.

[5] J. Gross, J. H. Highberger, and F. O. Schmitt, *Proc. Soc. Exptl. Biol. Med.*, **80**, 462, 1952.

[6] M. B. McEwen and M. I. Pratt, in J. T. Randall (ed.), *Nature and Structure of Collagen*, New York: Academic Press, 1953).

[7] P. Gallop, doctoral dissertation, Massachusetts Institute of Technology, 1953.

[8] The terminology which we have been using for some years to describe the various hierarchies of fibrous structures is as follows: the histologically observable fibrous unit of connective tissue is called the "fiber"; the structures observable in the electron microscope as the unit which is cross-striated and usually 500–1,000 A wide is called the "fibril"; when fibrils of collagen are treated with acid, they fray into thinner bundles, the "filaments"; the smallest fibrous units which show all the properties of collagen are called "protofibrils"—they may be one or only a few polypeptide chains in thickness.

[9] We are indebted to Dr. Karl Schmid for the preparation of purified α-1 acid glycoprotein obtained from human plasma.

[10] Glycine was determined by Christenson, Riggs, and Ray's (*Anal. Chem.*, **23**, 1521, 1951) modification of the method of Alexander, Landwehr, and Seligman (*J. Biol. Chem.*, **160**, 51, 1945), and hydroxyproline by Martin and Axelrod's (*Proc. Soc. Exptl. Biol. Med.*, **83**, 461, 1953) modification of the method of Neuman and Logan (*J. Biol. Chem.*, **184**, 299, 1950).

[11] The experiment was performed in this manner because lyophilized SLS is only partially soluble in neutral or slightly alkaline salt and buffer solutions and essentially insoluble in dilute acid. An aliquot of lyophilized SLS served to indicate the weight of the starting material. The conversion was performed with the second aliquot of wet SLS. It should be noted that excessive packing of an SLS precipitate by centrifugation will reduce its solubility.

[12] R. D. Harkness, A. M. Marko, H. M. Muir, and A. Newberger, *Biochem. J.*, **56**, 558, 1954.

[13] P. Vanamee and K. R. Porter, *J. Exptl. Med.*, **94**, 255, 1951.

[14] J. T. Randall, in J. T. Randall (ed.), *Nature and Structure of Collagen* (New York: Academic Press, 1953), p. 234.

[15] R. S. Bear, *J. Am. Chem. Soc.*, **64**, 727, 1942; *Advances in Protein Chem.*, **7**, 69, 1952.

[16] F. O. Schmitt, C. E. Hall, and M. A. Jakus, *J. Cellular Comp. Physiol.*, **20**, 11, 1942.

[17] F. O. Schmitt, C. E. Hall, and M. A. Jakus, *J. Appl. Phys.*, **16**, 263, 1945; F. O. Schmitt and J. Gross, *J. Am. Leather Chemists' Assoc.*, **43**, 458, 1948.

---

*ON THE GLUTAMATE-PROLINE-ORNITHINE INTERRELATION IN NEUROSPORA CRASSA**

BY HENRY J. VOGEL AND DAVID M. BONNER

DEPARTMENT OF MICROBIOLOGY, YALE UNIVERSITY

Communicated by Joseph S. Fruton, June 12, 1954

The metabolic relationship involving glutamate, proline, and ornithine has attracted the attention of a number of investigators working with a variety of species. With respect to mammals, the details of this relationship have been lucidly analyzed by Stetten.[1] In *Escherichia coli* an interrelation has been demonstrated[2] which differs substantially from that described by Stetten for mammals. A relationship involving these three amino acids has also been recognized in *Penicillium* and *Neurospora;* however, in these organisms the details are still obscure. Thus, Bonner suggested that proline and ornithine are formed from glutamate via a com-

mon precursor,[3] and Srb, Fincham, and Bonner proposed that the common precursor may be α-amino-δ-hydroxyvaleric acid or a chemical relative.[4] Fincham postulated that in *Neurospora* proline gives rise to ornithine and also considered the possibility that ornithine may form proline.[5] It was also suggested[6] that in *Neurospora* glutamic γ-semialdehyde (which is in equilibrium with the cyclized Δ^1-pyrroline-5-carboxylate) is a precursor of proline, since certain *Neurospora* mutants respond alternatively to proline or to glutamic γ-semialdehyde.[2, 6, 7]

The present investigation was undertaken to clarify the glutamate-proline-ornithine interrelation in *Neurospora* and to furnish information on the comparative biochemistry of these amino acids. The results obtained provide detailed evidence for metabolic paths leading from glutamate to proline, from glutamate to ornithine, and from exogenous ornithine to proline. In these three paths glutamic γ-semialdehyde is a key intermediate, as illustrated in Figure 1.

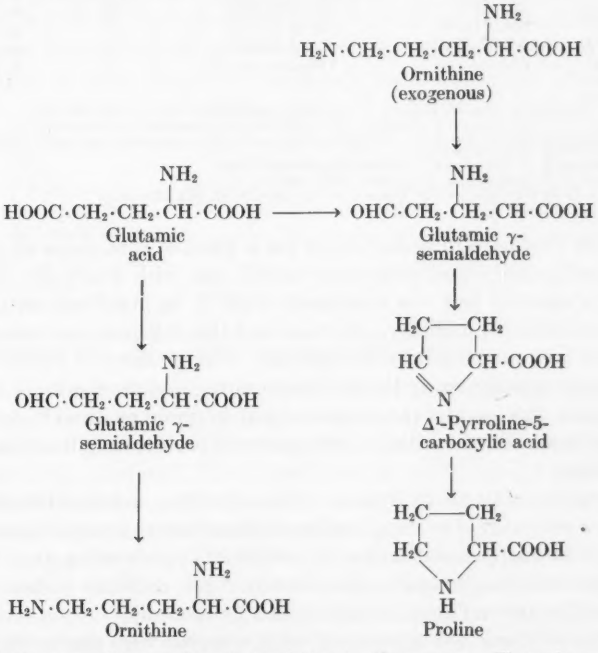


FIG. 1.—Glutamate-proline-ornithine interrelation in *Neurospora*. Glutamic γ-semialdehyde is shown twice to emphasize the possibility that the semialdehyde as proline precursor does not form a common pool with the semialdehyde as ornithine precursor. See text. The arrows shown are in the direction of biosynthesis.

These studies were primarily based on the ability of mycelial pads of proline- or ornithine-requiring mutants to excrete relevant products upon incubation with appropriate supplements, and also on growth responses of such mutants. The characteristics of the mutant strains used are summarized in Table 1.

For the mycelial-pad experiments, the mutants were grown in 125-ml. Erlenmeyer flasks containing 20 ml. of minimal medium[8] and an appropriate supplement (1 mg. of L-arginine hydrochloride for strain B or 2 mg. of L-proline for strain A; see

Table 1). After inoculation with conidia of the desired strain and incubation at 30° C. for about 40 hours without shaking, the mycelial pads obtained were gently lifted out of the flasks, rinsed by suspending in distilled water, resuspended in 3.5 ml. of 0.1 *M* phosphate at pH 7 (or pH 4.5, as indicated), and incubated with shaking at 30° C. for about 3 hours to permit depletion of endogenous substrates. Without renewal of the medium, the desired substrates were then added in a volume of 0.5–1 ml., and incubation with shaking at 30° was continued for an additional 8 hours. The mycelia were then removed, and the supernatant media were tested qualitatively and quantitatively for specific products formed.

TABLE 1

MUTANT STRAINS OF *Neurospora crassa*

Present Designation	Source Designation	Reference*	Source†	Growth Requirement‡
Strain A	21863-49A	Re of 21863§	I	p
Strain B	29997R1	Re of 29997‖	II	o, c, a
Strain C	35401R1A	Re of 35401#	II	g, p, o, c, a
Strain D	44207-P787-4a	Re of 44207#	II	g, p, o, c, a
Strain E	51506-T6a	Original strain#	I	g, p, o, c, a

* Re = reisolate.
† I = kindly furnished by Mrs. M. Bonner; II = kindly furnished by Mrs. M. B. Mitchell.
‡ *p* = proline; *o* = ornithine; *c* = citrulline; *a* = arginine; *g* = glutamic γ-semialdehyde. The growth requirements are satisfied by any one of the substances indicated. The requirement of strain A is not satisfied by *g*, *o*, *c*, or *a*; that of strain B, not by *g* or *p*.
§ G. W. Beadle and E. L. Tatum, *Am. J. Botany*, **32**, 678–686, 1945.
‖ A. M. Srb and N. H. Horowitz, *J. Biol. Chem.*, **154**, 129–139, 1944.
A. M. Srb, J. R. S. Fincham, and D. Bonner, *Am. J. Botany*, **37**, 533–538, 1950.

Ornithine to Proline.—In order to test for a possible conversion of ornithine to proline, mycelial-pad experiments were carried out with strain B. In a typical experiment a mycelial pad was incubated at pH 7, as described, with 7.5 micromoles of L-ornithine per milliliter. It was found that 0.9 micromole of L-proline per milliliter had been excreted into the medium. The presence of proline was recognized by bioautography, using the proline-requiring mutant strain 55-1 of *E. coli*.[6] The same strain was used for quantitative assay in liquid medium.[6] In control experiments without added ornithine, only traces of proline were found in the supernatant medium.

Glutamic γ-Semialdehyde to Proline.—Since glutamic γ-semialdehyde had been proposed as a precursor of proline,[6] analogous experiments were performed with the semialdehyde as a supplement instead of ornithine. Again using strain B, 8 micromoles of synthetic DL-glutamic γ-semialdehyde[6] per milliliter yielded 0.3 micromole of L-proline per milliliter, as determined by bioassay.[6]

Glutamate to Glutamic γ-Semialdehyde.—Experiments were also carried out to explore the possible conversion of glutamate to glutamic γ-semialdehyde. Strain A was used, because it does not give a growth response to glutamic γ-semialdehyde and might therefore be expected to excrete this compound. A mycelial pad of this strain was incubated at pH 4.5 with 50 micromoles of L-glutamate (previously adjusted to pH 4.5) per milliliter and 4 micromoles of *o*-aminobenzaldehyde per milliliter. The *o*-aminobenzaldehyde was employed as a trapping agent for glutamic γ-semialdehyde. In the supernatant medium glutamic γ-semialdehyde indeed accumulated, as indicated by the appearance of the previously described yellow color with the trapping agent.[6] Bioassay with *E. coli* mutant 55-25[6] showed that 0.5 micromole of L-glutamic γ-semialdehyde had been excreted per milliliter of super-

natant medium. No significant amounts of proline were found in the medium on testing with strain 55-1. Only traces of the semialdehyde were detected when either glutamate or the trapping agent was omitted.

Ornithine to Glutamic γ-Semialdehyde.—Since both ornithine and glutamic γ-semialdehyde were found to yield proline, the possible conversion of ornithine to glutamic γ-semialdehyde was examined. That this conversion can occur was indicated by Fincham's finding of an ornithine δ-transaminase.[5] In resting-pad experiments with strain A, 7.5 micromoles of L-ornithine per milliliter gave rise to 0.4 micromole of L-glutamic γ-semialdehyde per milliliter.

Selective Inhibition of Glutamic γ-Semialdehyde Formation.—In so far as both glutamate and ornithine can yield glutamic γ-semialdehyde, experiments were conducted to determine whether or not the conversion of glutamate to glutamic γ-semialdehyde involves ornithine as an intermediate. Again, using mycelial pads of strain A, the inhibitory effect of semicarbazide was examined, since it seemed possible that this effect might be selective (in view of the likelihood that ornithine δ-transaminase is pyridoxal phosphate–dependent and hence semicarbazide-sensitive). As seen in Table 2, semicarbazide at a concentration of 1 micromole per milliliter does not affect semialdehyde formation from glutamate; however, semialdehyde formation from ornithine is inhibited to the extent of 75 per cent. At a semicarbazide concentration of 4 micromoles per milliliter, the conversion of ornithine is completely inhibited, while semialdehyde is still formed from glutamate, albeit in reduced amounts. These data thus suggest that the conversion of glutamate to semialdehyde does not proceed via ornithine.

TABLE 2

SELECTIVE EFFECT OF SEMICARBAZIDE ON GLUTAMIC γ-SEMIALDEHYDE FORMATION BY MYCELIAL PADS*

Substrate†	Semicarbazide†	Glutamic γ-Semialdehyde†
Glutamate, 50	0	0.5
Glutamate, 50	1	0.5
Glutamate, 50	4	0.2
Ornithine, 7.5	0	0.4
Ornithine, 7.5	1	0.1
Ornithine, 7.5	4	0.0

* Proline-requiring *Neurospora* mutant strain A was used. See text for details.
† All the figures in this table represent concentrations in micromoles per milliliter.

Evidence against Proline as Ornithine Precursor.—Since in mycelial-pad experiments no evidence could be obtained for a conversion of proline to ornithine, experiments were done to determine whether such evidence could be adduced by means of another technique. Accordingly, growth tests (cf. Srb *et al.*[4]) were performed with strain A, using mixed supplements of proline and ornithine. When this strain was grown on limiting amounts (0.03–0.1 micromole per milliliter) of proline, addition of L-ornithine hydrochloride (0.03–0.1 micromole per milliliter) had no sparing or stimulating effect on growth. These findings suggest that proline does not readily yield ornithine and therefore probably is not a quantitatively important ornithine precursor. These conclusions thus render unlikely as a major path the cyclic scheme (with proline as ornithine precursor) which had been proposed for mammals by Shemin and Rittenberg[9] and for *Neurospora* by Fincham.[5]

Accumulation of α-Ketoisovalerate by Alternative Proline or Ornithine Responders.—During an investigation of the characteristics of strains C, D, and E (see Table 1),

which respond alternatively to proline, ornithine, citrulline, or arginine,[4] or glutamic γ-semialdehyde,[2,6,7] it was noted that these strains accumulate in their culture filtrates unusually large amounts of keto acids. Among these, α-ketoisovalerate was identified and estimated to occur in concentrations of 50–100 mg. per liter of culture filtrate. The identification of α-ketoisovalerate was based on paper chromatography,[10] combined with inhibition of the K-12 strain of *E. coli*,[11] and on the properties of the 2,4-dinitrophenylhydrazone of this keto acid, including melting point (199° C.), mixture melting point, and infrared spectrum.[12] The relationship, if any, between α-ketoisovalerate accumulation and the growth requirements of these mutants has not yet been analyzed.

Conclusions and Discussion.—The foregoing mycelial-pad experiments provide evidence for the following pathways:

a) Glutamate → glutamic γ-semialdehyde → Δ^1-pyrroline-5-carboxylate → proline.

b) Ornithine → glutamic γ-semialdehyde → Δ^1-pyrroline-5-carboxylate → proline.

c) Glutamate → glutamic γ-semialdehyde → ornithine.

Path *c* is indicated by the present finding that glutamate can form glutamic γ-semialdehyde, together with the earlier one by Fincham[5] that *Neurospora* has an enzyme which can convert the semialdehyde to ornithine.

In an effort to ascertain the physiological significance of these three paths, the characteristics of various relevant mutant strains were considered.

One class of mutants (strains C, D, and E) was shown to respond alternatively to proline, ornithine, citrulline, or arginine[4] and was subsequently found to respond also to glutamic γ-semialdehyde.[2, 6, 7] It was previously suggested[4] that such mutants are blocked in the synthesis of a common precursor of proline and of ornithine (which, in turn, is known to form arginine via citrulline[13]). The alternative response to proline or ornithine was thought to be due to a ready interconvertibility of these two amino acids via the common precursor, so that either amino acid could satisfy the requirement for both. The present results, which show that ornithine is readily converted to proline but not proline to ornithine, suggest that the interpretation of the behavior of these mutants needs reconsideration. Moreover, these results indicate that the deficiency in mutants such as strains C, D, and E is specifically in the synthesis of proline and not in that of arginine. Consistent with this conclusion are the growth responses of these and other mutants, as recorded by Srb *et al.*[4] Their data show that for mutants such as strain B (which respond to ornithine, citrulline or arginine but not to proline, and hence appear to have an arginine requirement) citrulline is a much more effective growth factor than ornithine. In contrast, for mutants such as strains C, D, and E, ornithine is a far better growth factor than citrulline. In the latter class of mutants, added ornithine therefore probably satisfies a requirement other than one for arginine. In view of the known convertibility of ornithine to glutamic γ-semialdehyde, the requirement satisfied by ornithine is presumably one for proline, with intermediate formation of glutamic γ-semialdehyde. The response to added glutamic γ-semialdehyde is also consistent with this interpretation, and so is the response to citrulline or arginine, since the latter two amino acids are known to give rise to ornithine via the "ornithine cycle".[13] The ornithine so formed may function as a source of glutamic γ-semialdehyde, as mentioned before.

It thus appears that mutants such as strains C, D, and E can make ornithine for arginine synthesis but nevertheless have a requirement for proline. Consequently, this endogenous ornithine apparently is not available for adequate proline synthesis. Similarly, if path *c* is a quantitatively substantial one, glutamic γ-semialdehyde as ornithine precursor does not seem to be available for adequate proline formation. It may therefore well be that *Neurospora* is organized in such a manner that the paths of proline and ornithine synthesis are physically separated. It is suggested that such a separation may be caused by a spatial organization of relevant enzyme systems,[14] with a resulting more or less restrictive "channeling" of metabolites.

The present interpretation of mutants such as strains C, D, and E also has a bearing on the evaluation of the significance of path *a*. It has been concluded that in these mutants glutamic γ-semialdehyde satisfies a proline requirement. This conclusion and the fact that proline-requiring strain A does not give a growth response to glutamic γ-semialdehyde but can excrete this substance in pad experiments indicate that *a* is a quantitatively major endogenous pathway.

The available information does not permit an evaluation of the quantitative significance of path *c*, leading to ornithine. The known genetically different ornithine-requiring mutants such as strain B and others[13] have been of little help in clarifying ornithine synthesis; on the other hand, the present results may aid in understanding the nature of these mutants. In so far as ornithine has been concluded to participate in a channeled pathway, the possibility merits consideration that these mutants are blocked through a disturbance in enzyme organization rather than through the actual absence (cf. Fincham[5]) of an enzyme. Such a disturbance could effect either the formation of ornithine or its utilization in the synthesis of arginine via citrulline.

The conclusions drawn here, from a study of *Neurospora* mutants, with respect to pathways as well as to channeling are fully consistent with metabolic relationships deduced from tracer experiments with *N. crassa* and *Torulopsis utilis* wild-type strains.[15]

From the point of view of comparative biochemistry, the picture of the glutamate-proline-ornithine interrelation now proposed for *Neurospora* is different from that found in *E. coli*[2] but is strikingly similar to that reported for mammals.[1] In *E. coli* ornithine is formed via several acetylated intermediates[2] and, when supplied exogenously, is converted to proline only to a minor extent.[16] In *Neurospora*, on the other hand, no evidence could be found for the participation of such acetylated intermediates,[2,17] and exogenous ornithine is readily converted to proline. Thus, although all these organisms utilize glutamate, proline, and ornithine as metabolites, the pathways associated with these metabolites show differences. Such instances of biochemical diversity should prove useful in the study of taxonomical and evolutionary relationships. In this connection it seems especially interesting that the fungus *Neurospora* more closely resembles mammals than it does the bacterium *E. coli*.

Summary.—In *N. crassa* proline is mainly synthesized from glutamate via glutamic γ-semialdehyde and Δ^1-pyrroline-5-carboxylate. Another route to proline proceeds from exogenous ornithine via glutamic γ-semialdehyde and Δ^1-pyrroline-5-carboxylate.

Evidence for the sequence glutamate–glutamic γ-semialdehyde–ornithine has been obtained, but the quantitative significance of this path is unknown.

Neurospora mutants responding alternatively to proline, ornithine, or glutamic γ-semialdehyde appear to be blocked between glutamate and glutamic γ-semialdehyde as proline precursor. Such mutants accumulate keto acids, among which α-ketoisovaleric acid has been identified.

A mutant responding to proline but not to glutamic γ-semialdehyde presumably is blocked in the conversion of the semialdehyde to proline.

Evidence has been presented which indicates that certain of the metabolites involved in the glutamate-proline-ornithine interrelation may be "channeled" as a result of the spatial organization of relevant enzyme systems.

The possibility has been considered that certain *Neurospora* mutants responding to ornithine but not to proline may be blocked through a disturbance in enzyme organization.

The glutamate-proline-ornithine interrelation in *Neurospora* is strikingly similar to that in mammals but differs from that in *E. coli*.

* This investigation was supported in part by a fellowship (to H. J. V.) from the Damon Runyon Memorial Fund, and in part by a grant (to D. M. B.) from the Atomic Energy Commission, Contract No. AT-(30-1)-1017. Preliminary note: H. J. Vogel, *Bacteriol. Proc.*, p. 101, 1954.

[1] M. R. Stetten, *J. Biol. Chem.*, **189**, 499–507, 1951. See this reference for earlier contributions.

[2] H. J. Vogel, these PROCEEDINGS, **39**, 578–583, 1953.

[3] D. Bonner, *Am. J. Botany*, **33**, 788–791, 1946.

[4] A. M. Srb, J. R. S. Fincham, and D. Bonner, *Am. J. Botany*, **37**, 533–538, 1950.

[5] J. R. S. Fincham, *Biochem. J.*, **53**, 313–320, 1953.

[6] H. J. Vogel and B. D. Davis, *J. Am. Chem. Soc.*, **74**, 109–112, 1952.

[7] N. Good and H. K. Mitchell, *J. Am. Chem. Soc.*, **74**, 4952–4953, 1952; A. M. Srb and J. R. S. Fincham, cited in Fincham, *op. cit.*

[8] F. J. Ryan, G. W. Beadle, and E. L. Tatum, *Am. J. Botany*, **30**, 784–799, 1943.

[9] D. Shemin and D. Rittenberg, *J. Biol. Chem.*, **158**, 71–76, 1945.

[10] W. K. Maas and H. J. Vogel, *J. Bacteriol.*, **65**, 388–393, 1953.

[11] E. L. Tatum, *Cold Spring Harbor Symposia Quant. Biol.*, **11**, 278–284, 1946.

[12] Infrared spectra of the 2,4-dinitrophenylhydrazone derived from the natural product and of α-ketoisovaleric acid 2,4-dinitrophenylhydrazone were kindly compared and found identical by Drs. H. H. Wasserman and D. D. Grassetti.

[13] A. M. Srb and N. H. Horowitz, *J. Biol. Chem.*, **154**, 129–139, 1944.

[14] For a review on organized enzyme systems see D. E. Green, *J. Cellular Comp. Physiol.*, **39** (Suppl. 2), 75–111, 1952. This review deals primarily with particulate systems. The term "organization" as used here is not intended to be necessarily restricted to so-called "particle-bound" enzymes.

[15] P. H. Abelson and H. J. Vogel (to be published).

[16] H. J. Vogel (to be published).

[17] No acetylornithinase activity could be detected in extracts of wild-type *N. crassa* by means of experiments analogous to those which permitted the demonstration of this enzyme in *E. coli*; cf. Vogel (1953), *op. cit.*

*CAROTENOID DIFFERENCES IN LYCOPERSICON: HYBRIDS OF AN UNUSUAL RACE OF L. PIMPINELLIFOLIUM**

By G. Mackinney, C. M. Rick, and J. A. Jenkins

DEPARTMENTS OF FOOD TECHNOLOGY, VEGETABLE CROPS, AND GENETICS, UNIVERSITY OF CALIFORNIA, BERKELEY AND DAVIS

Communicated by R. E. Clausen, June 15, 1954

The wild tomato, *Lycopersicon pimpinellifolium*, bears diminutive fruits that are normally red-pigmented, the carotenoid content being similar to that of the cultivated tomato, *L. esculentum*. A strain of the former, recently collected on Indefatigable Island of the Galapagos archipelago, has orange-colored fruits. Its carotenoid pigmentation may consist almost entirely of β-carotene,[1] in place of all-*trans* lycopene, the principal pigment of the red-fruited varieties. In this respect, it clearly resembles the β-orange tomato segregant from hybrids of *L. hirsutum* × *L. esculentum* isolated by Lincoln and Porter[2] and further discussed by Tomes *et al.*[3, 4]

The Galapagos accession was received by C. M. Rick from Mr. Alf Kastdalen, a Norwegian settler. It has also been collected more recently from the same site by Mr. Robert Bowman, of the Department of Zoölogy, University of California, Berkeley. Both collectors, to whom we are greatly indebted for this material, observed the same orange fruit color of plants which were growing in their native habitat. A specimen from the Gray Herbarium, which was collected as No. 3375 by Alban Stewart on the southeast side of Indefatigable Island in 1905, appears identical with our material in all determinable characteristics.

Except for its anomalous fruit color and the presence of a few large hairs on the upper stem, the Galapagos collection conforms to descriptions of *L. pimpinellifolium*. This classification is confirmed by breeding tests; it is fully compatible with other strains of this species and also with *L. esculentum*, and F_1 hybrids of such crosses are fully fertile; on the other hand, it will not cross with other species of the genus without the aid of special techniques.

Methods.—The Galapagos accession was hybridized with Pearson, a large red-fruited variety of *L. esculentum*. The resulting F_1 hybrid was backcrossed to Pearson and also selfed for the production of an F_2 generation. The parent, hybrid, and segregating generations were grown under uniform conditions in the field at Davis in the Sacramento Valley.

Methods of extracting the carotenoids from the fruit and preparing the extracts for spectrophotometric and chromatographic analyses have been discussed previously.[5] We have not hitherto emphasized the xanthophyll fraction, which in many cases has not been detected on the chromatogram; or, where this is the case, traces of green would lead to the supposition that xanthophylls would naturally be associated with the chlorophylls in fruit that is not fully ripe. This, however, is not adequate to explain the xanthophyll fraction found in some red- and yellow-fruited strains. When there is a clear orange zone on the chromatogram, above the lycopene, it is removed from the column, eluted with acetone, and the absorption maxima of the eluate determined. These usually fall between 470 and 475 mμ (peak I) and 440 and 445 mμ (peak II). An absorption coefficient of 250 (in liters per gram centimeter) at the second peak is assumed, and the concentration of

"total" xanthophyll is thus computed. The other components are estimated as previously described.

In two cases the Berkeley-grown Galapagos fruit contained sufficient chlorophyll that a cold saponification with 95 per cent alcoholic potassium hydroxide solution was necessary. In these instances, the extracts, *ca.* 25 ml., were shaken with 20 ml. alcoholic hydroxide for 3–5 minutes, diluted with water, and the aqueous phase discarded. The extract, in petroleum ether, was further washed with water, dried as before, and the clear solution made to volume for spectrophotometric and chromatographic analyses.

Discussion of Results.—Carotenoid analyses of representative fruits from the parental and hybrid generations are presented in Table 1. In addition to the parents

TABLE 1

CAROTENOID CONTENTS OF FRUITS FROM INDIVIDUAL PLANTS (IN MICROGRAMS PER GRAM OF FRESH FRUIT)

Type*	Lycopene	β-carotene	ζ-carotene	Phytofluene	Xanthophylls
L. pimpinellifolium:					
Galapagos	...	75.6	...	5.6	13.2
Galapagos (Berkeley) †	...	3.2	...	Trace	15.9
Galapagos (Berkeley) ‡	...	2.9	...	Trace	19.2
Red	85.4	9.3	...	2.0	...
L. esculentum var. Pearson:					
Red	78.0	10.3	...	6.1	Traces
	77.1	13.1	...	7.5	Traces
	86.8	6.9	...	Not det.	Traces
F_1 Pearson × Galapagos:					
Orange	9.5	48.1	...	5.0	Not det.
	5.3	46.2	...	5.3	6.3
F_2 Pearson × Galapagos:					
Yellow and orange	0.6	41.2	...	6.2	9.0
	0.6	39.9	...	2.2	9.8
	1.4	28.2	...	6.4	5.2
	9.9	45.3	Trace	3.9	6.2
	7.4	43.8	...	5.5	9.1
	5.0	53.5	...	2.4	Not det.
Orange-red	24.7	33.5	11.0	Not det.	4.7
	35.9	47.7	7.5	4.5	8.9
Red	55.2	7.4	4.9	6.6	4.7
	69.6	2.0	2.7	6.4	3.1
	65.7	16.0	3.4	8.9	9.3
	42.7	4.6	Not det.	4.4	5.6
B_1 (Pearson × Galapagos) × Pearson:					
Orange	6.3	42.7	...	4.0	16.6
	2.4	49.2	...	2.7	10.8
Red	85.5	12.7	1.7	8.6	Trace
	82.8	7.4	6.9	14.3	Trace

* All were grown at Davis unless otherwise noted.
† Held for 2 weeks after picking.
‡ Analyzed at once.

used in the cross, *L. esculentum* var. Pearson and the orange-fruited accession of *L. pimpinellifolium* from the Galapagos Islands, we have included a typical red-fruited form of *L. pimpinellifolium*. The latter has fruits weighing about 1 gm., which are essentially the same size as those of the Galapagos accession. Pearson, on the other hand, has large fruits weighing about 200 gm. each. Nevertheless, the two red-fruited forms have about the same carotenoid concentration, on a fresh-weight basis.

Unfortunately, seeds of the Galapagos accession are extremely difficult to germinate under our conditions, and low germination was also characteristic of the F_2 generation, which made the phenotypic ratios obtained completely meaningless. In contrast, the F_1 and backcross generations were normal in respect to germination. A second difficulty was encountered with the Galapagos parent and certain of the F_2 segregants. Whereas Pearson, red-fruited *L. pimpinellifolium*, F_1, backcross, and the red and orange segregants of the F_2 hybrids develop their characteristic colors rapidly as they mature, the Galapagos race and certain yellow-fruited F_2 segregants turn color much more slowly. Fruits of the Galapagos parent that were grown at Davis during the summer became buff-orange on losing their chlorophyll. When grown in the greenhouse during the winter, the fruit was less intensely colored. At Berkeley fruits that ripened in the greenhouse during the spring had a definite greenish-yellow color at maturity, and the extract spectrum resembled that of a typical yellow-fruited tomato (rrt^+t^+), which is characteristically low in β-carotene. The greenish cast did not disappear on holding the fruits at 20°–25° C. for two weeks after picking. Nevertheless, under the conditions at Davis, where all the hybrid generations were grown, a high β-carotene content can be expressed in the Galapagos parent.

For simplicity, we have omitted data on γ-carotene from Table 1. This carotenoid was not detected in either type of *pimpinellifolium*. It appeared in highest amount (*ca.* 5 μg. per gram) in Pearson and in trace amounts up to 1.9 μg. per gram in the following: the F_1 hybrid, red segregates of the F_2, and both of the two classes in the backcross. Xanthophylls, on the other hand, were significantly higher in the Galapagos parent than in Pearson, and in the segregating generations they were found in largest amounts in the yellower or more Galapagos-like classes. In contrast to γ-carotene and xanthophylls, ζ-carotene was not detected in either race of *pimpinellifolium* or in the F_1 hybrid, and its presence in Pearson was doubtful. Nevertheless, it did appear in both the F_2 and the backcross and, interestingly enough, in the more red or Pearson-like segregates of these two generations. The amount of phytofluene varied as much within classes as between classes; that is, there was no consistent pattern in its distribution. Finally, it is clear from Table 1 that the main interest lies in the reciprocal variation of lycopene and β-carotene. The Galapagos parent showed no ability to synthesize lycopene at all, while, under favorable conditions, its content of β-carotene was exceedingly high. Pearson showed the reverse, with lycopene as its major component.

The pattern of variation in lycopene and β-carotene is strongly reminiscent of that attributed to segregation at the *B* locus by Lincoln and Porter.[2] The evidence is particularly strong in the case of the backcross to Pearson. There was a clear-cut one-to-one segregation: 26 red-fruited plants to 25 with orange fruit. The former resembled Pearson in color and carotenoid content; the latter were similar to the F_1 hybrid. These results indicate that a single gene of major effect controls the segregation of fruit color in this backcross. Further evidence is provided by the observation in the same progeny that all red-fruited plants were determinate (*sp/sp*, as in Pearson) and all orange-fruited plants were indeterminate (*sp/+*, as in the F_1). In other words, a single gene or gene complex closely linked with the self-pruning locus must be responsible for this segregation of fruit color. While the orange segregates in the backcross clearly resemble the F_1 in appearance and in their content of ly-

copene and β-carotene, both types have a lower lycopene content than Lincoln and Porter observed in their $B/+$ genotype.

Three phenotypic classes were observed in the F_2 generation, but, as mentioned above, their ratio of occurrence could not be established, owing to poor germination and, at times, lack of fruiting. In the first class the fruits were either yellow or orange and not unlike the Galapagos parent, except that they all contained some lycopene and their content of β-carotene was definitely lower. The orange fruits in this class were similar to the F_1 both in appearance and in their content of lycopene and β-carotene. Fruits in the second class had a distinct orange-red color and were more or less intermediate to the parents in their lycopene and β-carotene content. However, they contained much more lycopene than the F_1. Finally, the third class had red fruits like Pearson but lacked the high lycopene content of the latter.

It is significant that in the F_2 no segregants recovered were able to synthesize as much β-carotene as the Galapagos parent, and, at the other extreme, no segregants were able to synthesize as much lycopene as Pearson. Furthermore, no F_2 segregant was completely devoid of lycopene, as was the Galapagos parent under our conditions. We have remarked before that the ripening behavior of fruits of Pearson and the Galapagos race are quite different, and the possibility exists that, in its natural habitat, the latter might be capable of producing small amounts of lycopene, as in the lightest-colored F_2 segregants. However, we are inclined to attribute the failure to obtain the parental types in the extremes of the F_2 segregation to segregation at other loci, which would make the probability of recovering parental types much smaller.

Segregation of modifying genes at one or more other loci may also be responsible for another aspect of the F_2 segregation. If the orange segregants of the first class are heterozygous on a single-gene basis and are similar to the F_1, then the orange-red class is clearly anomalous. Furthermore, modifying genes could be responsible for minor variation in some of the pigment. For example, the red segregants in F_2 had a β-carotene content ranging from 2.0 to 16.0 μg., and the reds in the backcross ranged from 7.4 to 12.7 μg. The inhibitor postulated by Tomes and Quackenbush[4] may possibly be involved, but any modifier complex proposed must also account for the clear-cut segregation in the backcross to Pearson in the present case.

In spite of the anomalous behavior of the F_2 generation, we are inclined, in view of the backcross data, to believe that a single gene of major effect is segregating in this material. It is similar to, if not identical with, the *B* gene introduced from *L. hirsutum* into *L. esculentum* by Lincoln and Porter.[2] However, since the presence of the *B* allele in Galapagos accession of *L. pimpinellifolium* has not been unequivocally established, a symbol is unnecessary at this juncture.

Summary.—An unusual race of orange-fruited *L. pimpinellifolium* from the Galapagos Islands has been examined for carotenoid differences. Unlike its red counterpart on the mainland, which synthesizes primarily all-*trans* lycopene, this fruit may contain predominantly β-carotene. We have included data on the carotenoid content of fruits of the F_1 hybrid with *L. esculentum* (Pearson) and segregants of the F_2 and backcross to Pearson. Evidence was obtained for the presence in the Galapagos accession of a gene that exerts a major effect on carotenoid content and is tightly linked with the self-pruning (*sp*) locus.

* This work was supported in part by research grant NSF-G420 of the National Science Foundation.

[1] The Berkeley-grown fruit resembles that of the yellow rrt^+t^+ genotype in carotenoid content. Many specimens have shown decidedly weak pigmentation, with relatively low concentrations of β-carotene. However, the potentiality for high β-carotene production is present.

[2] R. E. Lincoln and J. W. Porter, *Genetics*, **35**, 206, 1950.

[3] M. L. Tomes and F. W. Quackenbush, *Genetics*, **38**, 696–697, 1953.

[4] M. L. Tomes, F. W. Quackenbush, O. E. Nelson, Jr., and Betty North, *Genetics*, **38**, 117–27, 1953.

[5] G. Mackinney and J. A. Jenkins, these PROCEEDINGS, **35**, 284, 1949; *ibid.*, **38**, 48, 1952.

LIMIT THEOREMS FOR HOMOGENEOUS STOCHASTIC PROCESSES

BY S. BOCHNER

PRINCETON UNIVERSITY

Communicated May 17, 1954

We take a random-valued vector function

$$x(\Delta) = \{x_1(\Delta), \ldots, x_k(\Delta)\}, \tag{1}$$

which is defined on the *intervals* $\Delta: (\tau_1 < \tau \leq \tau_2)$ of the τ-axis and is additive, $x(\Delta' + \Delta'') = x(\Delta') + x(\Delta'')$; and we also take a point transformation

$$y_1 = \mu_1(x_1, \ldots, x_k), \ldots, \qquad y_l = \mu_l(x_1, \ldots, x_k)$$

from E_k to E_l which is continuous everywhere and maps the origin into the origin, $\mu(0) = 0$.

The interval function $\eta(\Delta) = \mu(x(\Delta))$ is not itself additive, and the natural way for securing additivity is to form a variation thus:

$$y(\Delta) = \lim_{n \to \infty} \text{in prob} \sum_{\nu=1}^{\nu_n} \mu(x(\Delta_{n\nu})), \tag{2}$$

where $\Delta = \Delta_{n1} + \ldots + \Delta_{n\nu_n}$ and $\nu_n \to \infty$. The Central Limit theory would suggest taking the limit of

$$b_n \sum_{\nu=1}^{\nu_n} \mu(x(\Delta_{n\nu})) - a_n$$

for suitable constants a_n, b_n, but we will do this only once, namely in Theorem IX and then only for $b_n = 1$.

For $l = 1$, if $\mu(x_j) = |x| \equiv (x_1^2 + \ldots + x_k)^{1/2}$ then $y(\Delta_0)$ is the length of the path (1) over the interval Δ_0 and for $\mu(x_j) = |x|^2$ the corresponding $y(\Delta_0)$ is the analogue to the χ^2-distribution, in a sense. If a numerical $x(\Delta)$ is continuous in Δ and if it has finite length, then the χ^2-variation is zero; but our random $x(\Delta)$ will not be so continuous (usually), and it will possess many different variations simultaneously, none of which will be 0 or $+\infty$ with positive probability.

In what follows we assume that $x(\Delta)$ is a homogeneous process, meaning that for each Δ the (joint) distribution function of formula (1) depends only on the

length t of Δ; and, if we denote it by $F(A; t)$ then there exists a function $\varphi(\alpha_1, \ldots, \alpha_k)$ such that

$$e^{-t\varphi(\alpha_j)} = \int_{E_k} e^{-2\pi i(\alpha,x)}\, dF(x;\ t), \qquad 0 < t < \infty. \tag{3}$$

Formally the function $y(\Delta)$ is of the same kind, and, if we denote its characteristic functions by

$$e^{-t\psi(\beta_1, \ldots, \beta)_s},$$

then the problem arises of linking the functions $\varphi(\alpha_r)$ and $\psi(\beta_s)$ by formulas, if possible. If in formula (2) the interval Δ is (0, 1) and the partintervals $\Delta_{n\nu}$ have equal length for each n, then equation (2) is equivalent with the limit relation

$$e^{-\psi(\beta_s)} = \lim_{n\to\infty} \left(\int_{E_k} e^{-2\pi i(\beta,\mu(x))}\, dF\left(x; \frac{1}{n}\right)\right)^n, \tag{4}$$

with n ranging over an infinite subsequence $\{n_\nu\}$ of all integers. Also, if we put

$$G_n(A) = nF\left(A; \frac{1}{n}\right), \tag{5}$$

then relation (4) becomes more or less equivalent with

$$\psi(\beta_1, \ldots, \beta_l) = \lim_{n\to\infty} \int_{E_k} (1 - e^{-2\pi i(\beta,\mu(x))})\, dG_n(x), \tag{6}$$

and in our theorems we will be concerned with this relation (6) only and not at all with the original relation (2) itself. Also, for our purposes, the dimension l of the image space can be reduced to 1 by putting

$$\beta_s = \beta\gamma_s, \qquad \mu(x_r) = \gamma_1\mu_1(x_r) + \ldots + \gamma_l\mu_l(x_r),$$

and, accordingly, we will be dealing with the relation

$$\psi(\beta) = \lim_{n\to\infty} \int_{E_k} (1 - e^{-2\pi i\beta\mu(x)})\, dG_n(x), \tag{7}$$

in which $G_n(A)$ is taken from equation (3) by way of equation (5), and $\mu(x_1, \ldots, x_k)$ is a function in E_k, continuous everywhere and 0 at the origin; and its continuity can, in fact, be generalized to Baire measurability with regard to the functions $G_n(A)$ given, if this be desired, except at the origin, where continuity is not easily dispensed with.

As known, the function $\varphi(\alpha_r)$ has a representation

$$\varphi(\alpha_r) = i\sum_{p=1}^{k} c_p\alpha_p + \sum_{p,\,q=1}^{k} c_{pq}\alpha_p\alpha_q + \int_{E_k'} \left(1 - e^{-2\pi i(\alpha,\,x)} - \frac{2\pi i(\alpha, x)}{1 + |x|^2}\right) dG(x), \tag{8}$$

and from the theory underlying it[1] the following theorems can be obtained:

THEOREM I.—If we have

$$\mu(x_1, \ldots, x_k) = 0(|x|), \qquad x \to 0,$$

then there is a subsequence $\{n_\nu\}$ for which expression (7) exists, and the limit function has a representation

$$\psi(\beta) = im\beta + \gamma\beta^2 + \int_{E_k'} \left(1 - e^{-2\pi i\beta\mu(x)} - \frac{2\pi i\beta u(x)}{1 + |\mu(x)|^2}\right) dG(x), \qquad (9)$$

in which the Poisson weight function $G(A)$ is the same as in equation (8), but m and γ are constants depending on the subsequence.

THEOREM II.—If

$$\mu(x_1, \ldots, x_k) = \sum_{p=1}^{k} a_p x_p + o(|x|), \qquad x \to 0,$$

then the value of γ is likewise fixed, namely,

$$\gamma = \sum_{p,\,q=1}^{k} c_{pq} a_p a_q,$$

and in particular for

$$\mu(x_1, \ldots, x_k) = 0(|x|), \qquad x \to 0,$$

we have $\gamma = 0$ (no Gaussian part in $\psi(\beta)$).

THEOREM III.—If

$$\mu(x_1, \ldots, x_k) = 0(|x|^2), \qquad x \to 0,$$

then we can write

$$\psi(\beta) = im\beta + \int_{E_k'} (1 - e^{-2\pi i\beta\mu(x)})\, dG(x)$$

without a corrective term in the integral.

THEOREM IV.—If

$$\mu(x_1, \ldots, x_k) = \sum_{p,\,q=1}^{k} a_{pq} x_p x_q + o(|x|^2), \qquad x \to 0,$$

then the entire sequence (7) converges and

$$\psi(\beta) = i\left(\sum_{p,\,q=1}^{k} c_{pq} a_{pq}\right)\beta + \int_{E_k'} (1 - e^{-2\pi i\beta\mu(x)})\, dG(x), \qquad (10)$$

and in particular for

$$\mu(x_1, \ldots, x_k) = o(|x|^2), \qquad x \to 0,$$

we have directly

$$\psi(\beta) = \int_{E_k'} (1 - e^{-2\pi i\beta\mu(x)})\, dG(x), \qquad (11)$$

without any corrective or additional term whatsoever.

We note that for $k = 1$ and $\mu(x) = x^2$ formula (10) was known.[2]

Continuing, we will for the present say that the given process (3) is *sharply Poisson* if, for any $\mu(x_j)$ for which

$$\int_{0 < |x| < 1} |\mu(x)|\, dG(x) < \infty, \qquad (12)$$

the limit (7) exists and the limit function has the pure form (11). The following sufficient criterion is obvious.

THEOREM V.—The process (3) is sharply Poisson if we have

$$G_1(A) \leq G_2(A) < \ldots \rightarrow G(A), \tag{13}$$

or, more generally,

$$G_n(A) \leq M \cdot G(A).$$

For a normal process we have $G(A) = 0$ and $G_n(A) \not\equiv 0$, and this violates condition (13) flagrantly. However, for a symmetric Cauchy process it is fulfilled, because then $G_n(A)$ is the indefinite integral of the point function

$$(n^{-2} + x_1^2 + \ldots + x_k^2)^{-(1/2)(k+1)},$$

except for a fixed factor, and this is monotonely increasing with n, obviously. Also any process subordinate[1] to it is likewise so, and thus, if we introduce the symmetric stable processes

$$\varphi(\alpha_j) \equiv |\alpha|^p \equiv c_p \int_{E_k'} \frac{(1 - \cos 2\pi(\alpha, x))\, dv_x}{|x|^{p+k}} \tag{14}$$

for $0 < p < 2$, then we obtain the following statement:

THEOREM VI.—For $0 < p \leq 1$, if

$$\int_{0 < x \leq 1} \frac{|\mu(x_1, \ldots, x_k)|\, dv_x}{|x|^{p+k}} < \infty,$$

then the entire sequence (7) converges, and we have

$$\psi(\beta) = c_p \int_{E_k'} \frac{(1 - e^{-2\pi i \beta \mu(x)})\, dv_x}{|x|^{p+k}}. \tag{15}$$

A related but different theorem,[3] which also applies to $1 < p < 2$, is as follows:

THEOREM VII.—If the given function has a pure representation

$$\varphi(\alpha_j) = \int_{E_k'} (1 - \cos 2\pi(\alpha, x))\, dG(x)$$

to start with, if $\mu(x_1, \ldots, x_k) \geq 0$ and $\mu(-x_1, \ldots, -x_k) = \mu(x_1, \ldots, x_k)$, if $e^{-\mu(x_1, \ldots, x_k)}$) is positive-definite and relation (12) holds, then the entire sequence (7) converges, and we have

$$\psi(\beta) = \int_{E_k'} (1 - \cos(2\pi\beta\mu(x)))\, dG(x).$$

This leads to the following conclusion:

THEOREM VIII.—For any process (14), if we choose $\mu(x_j) = |x|^q$, where

$$p < q < \infty,$$

then the entire sequence (7) converges, and we have

$$\psi(\beta) = c_{p,q} \lim_{\epsilon \rightarrow 0} (\epsilon + i\beta)^{p/q},$$

meaning that $\psi(\beta)$ describes a nonsymmetric stable process of the fractional exponent p/q.

In particular, if $x(\Delta)$ is a symmetric stable process of exponent $p < 1$, then almost all paths have finite length,[3] and the corresponding interval function $y(\Delta)$ is a (nonsymmetric) stable process of the same exponent p.

If we let $q \to p$, then $c_{p,q} \to \infty$, but the following statement remains, nevertheless.

THEOREM IX.—For any $0 < p < 2$ there are constants $a_{np} = c_p \log n + 0(1)$, $n \geq 3$, such that

$$\lim_{n \to \infty} \left[\int_{Ek'} \frac{1 - e^{-2\pi i \beta |x|^p}}{|x|^{p+k}} dv_x - a_{np} \right]$$

exists and has the value

$$\int_{Ek'} \left(1 - e^{-2\pi i \beta |x|^p} - \frac{2\pi i \beta |x|^p}{1 + |x|^{2p}} \right) dv_x \equiv c_p' i\beta \lim_{\epsilon \to 0} \log \frac{\epsilon + i\beta}{e},$$

which represents a quasi-stable process,[4] so-called.

For the Cauchy process $p = 1$ this means that a finite path length still exists and constitutes a quasi-stable process itself, provided that, for the computation of the length, we choose a fixed sequence of polygonal approximations common to all paths and at each step subtract a suitable constant number tending to $+\infty$, the same for all paths.

Remarks: If we start out with a general function (8), then formula (11) may be used to detach the Poisson weight function $G(A)$ from the Gauss-Bernoulli components, and by suitable choice of $\mu(x)$ various parts of $G(A)$ may be emphasized for purposes of computation and even estimation, perhaps. Also, if $G(A)$ is assumed known, then the Gaussian part may be obtained from formula (10), and the linear part may then be obtained from formula (8) in the end.

Furthermore, a good part of our analysis could also be upheld, with some care, for homogeneous processes in which the feature of stationarity has been attenuated to an extent. We assume that our interval function $x(\Delta)$ is such that for each Δ its characteristic function is of the form $\exp(-\psi(\alpha_j; \Delta))$, where each $\psi(\alpha_j; \Delta)$ is of the form

$$i \sum_p c_p(\Delta)\ \alpha_p + \sum_{p,q} c_{pq}(\Delta) \alpha_p \alpha_q + \int_{Ek'} \left(1 - e^{-2\pi i(\alpha, x)} - \frac{2\pi i(\alpha, x)}{1 + |x|^2} \right) dG(x; \Delta)$$

and possesses the additivity property

$$\psi(\alpha; \Delta' + \Delta'') = \psi(\alpha; \Delta') + \psi(\alpha; \Delta'')$$

but is not of the strictly linear form $|\Delta| \psi(\alpha)$, as heretofore. Its variation (2) has a similar structure and, again for $l = 1$, the exponent $\psi(\beta; \Delta)$ has again a representation

$$im(\Delta)\beta + \gamma(\Delta)\beta^2 + \int_{Ek'} \left(1 - e^{-2\pi i \beta \mu(x)} - \frac{2\pi i \beta \mu(x)}{1 + |\mu(x)|^2} \right) dG(x; \Delta),$$

and Theorems I, II, III, IV and VII can be adapted to this situation, subject to assumptions.

[1] See the book by S. Bochner, *Harmonic Analysis and the Theory of Probability* (to appear).

[2] K. Kunisawa and G. Maruyama, "Some Properties of Infinitely Divisible Laws," *Repts. Stat. Appl. Research Union Japan. Sci. Eng.*, **1**, No. 3, 22–27, 1951.

[3] S. Bochner, "Stochastic Processes," *Ann. Math.*, **48**, 1014–1060, 1947, in particular pp. 1031–1037; and "Length of Random Paths on General Homogeneous Spaces," *ibid.*, **57**, 309–313, 1953.

[4] P. Lévy, *Théorie de l'addition des variables aléatoires* (Paris, 1937).

*SUR LES GROUPES D'EILENBERG-MAC LANE. II**

By Henri Cartan

Université de Paris

Communicated by Saunders Mac Lane, May 18, 1954

1. *Les Algèbres d'Homologie* $H_*(\Pi, n; Z_p)$.—Π désigne un *groupe cyclique* (fini ou infini), Z_p le corps des entiers mod p (premier). On notera $E(m; \Lambda)$ la Λ-algèbre graduée de base $(1, x)$, x de degré m, avec $x^2 = 0$; x s'appelle le générateur de l'*algèbre extérieure* $E(m; \Lambda)$, à coefficients dans l'anneau Λ. On notera $P(m; \Lambda)$ la Λ-algèbre graduée de base $(1, x^{(1)}, x^{(2)}, \ldots, x^{(k)}, \ldots)$, $x^{(k)}$ de degré km, avec la multiplication

$$x^{(k)}x^{(h)} = \frac{(k+h)!}{k!h!}\, x^{(k+h)};$$

$x^{(1)} = x$ s'appelle le "générateur" de l'*algèbre des polynômes modifiée* $P(m; \Lambda)$. Les algèbres $E(m; \Lambda)$ et $P(m; \Lambda)$ sont *anticommutatives* (car on supposera, pour $P(m; \Lambda)$, que m est pair si Λ n'est pas de caractéristique 2).

Prenons $\Lambda = Z_p$. On a une "construction" (au sens de I, § 2), d'algèbre initiale $A = Z_p(\Pi)$, et dont voici l'algèbre finale N: si Π est cyclique infini, $N = E(1; Z_p)$ avec différentielle nulle; si Π est cyclique d'ordre p^f, $N = E(1; Z_p) \otimes {}_{Z_p}P(2; Z_p)$ avec différentielle nulle. Si Π est cylique d'ordre premier à p, l'algèbre N est acyclique, donc $H_*(\Pi, n; Z_p)$ est acyclique pour tout $n \geq 1$.

On cherche une *construction itérée* ayant comme algèbre initiale $E(1; Z_p) \approx H_*(Z, 1; Z_p)$, resp. $E(1; Z_p) \otimes P(2; Z_p) \approx H_*(Z_{p^f}, 1; Z_p)$. D'après I, §2, il suffit de faire une construction pour chaque facteur du produit tensoriel. Plus généralement:

I. *Construction ayant* $E(m-1; Z_p)$ *pour algèbre initiale* (m *pair*). Soit $A = E(m-1; Z_p)$, $N = P(m; Z_p)$; l'algèbre $M = A \otimes N$, munie de la différentielle d définie par $dx = 0$, $dy^{(h)} = xy^{(h-1)}$ (x générateur de A, y générateur de N), est acyclique; par passage au quotient, d est nul sur l'*algèbre finale* $P(m; Z_p)$. La *suspension* envoie x en y.

II. *Construction ayant* $P(m; Z_p)$ *comme algèbre initiale.* Soit x le "générateur" de $P(m; Z_p)$. Notons $Q_p(n)$ l'algèbre graduée, quotient de l'algèbre des polynômes $Z_p[u]$ par l'idéal (u^p), u de degré n. Soit f_k l'homomorphisme d'algèbres $Q_p(p^k m) \to P(m; Z_p)$ qui envoie le générateur u_k de $Q_p(p^k m)$ dans $x^{(p^k)}$. Les f_k identifient l'algèbre $P(m; Z_p)$ au produit tensoriel (infini) $\otimes_{k \geq 0} Q_p(p^k m)$. Soit $A_k = Q_p(p^k m)$; c'est l'algèbre initiale d'une construction: prendre $N_k = E(p^k m + 1; Z_p) \otimes P(p^{k+1}m + 2; Z_p)$ et, sur l'algèbre $M_k = A_k \otimes N_k$, la différentielle d définie par

$$du_k = 0, \qquad dy_k = u_k, \qquad dz_k{}^{(h)} = (u_k)^{p-1} y_k z_k{}^{(h-1)} \tag{1.1}$$

(y_k, z_k: générateurs de $E(p^k m + 1; Z_p)$, $P(p^{k+1}m + 2; Z_p)$). Par passage au quotient, d est nul sur N_k. Par produit tensoriel, on trouve une *construction d'algèbre initiale* $A = P(m; Z_p)$ *et d'algèbre finale* $N = \otimes_{k \geq 0} N_k$. La *suspension* envoie $x^{(p^k)} = u_k$ en y_k, et est nulle sur les autres éléments de la base de $P(m; Z_p)$.

Par itération des constructions I et II, l'algèbre d'homologie $H_*(\Pi, n; Z_p)$ (pour $\Pi = Z$ ou Z_{p^f}) est un produit tensoriel d'algèbres extérieures à un générateur de

degré impair, et d'algèbres de polynômes modifiées à un générateur de degré pair. Chaque générateur u de $H_*(\Pi, n; Z_p)$ a pour suspension un générateur de $H_*(\Pi, n+1; Z_p)$; u est dit *primitif* s'il n'est pas le suspendu d'un générateur de $H_*(\Pi, n-1; Z_p)$. Chaque générateur u de $H_*(\Pi, n; Z_p)$ est caractérisé par l'unique générateur primitif v dont il est le suspendu itéré; si $v \in H_*(\Pi, m; Z_p)$, m s'appelle l'*indice* de u. Si u est de degré $n+q$, q est le *degré stable* de u (invariant par suspension). L'ensemble de tous les générateurs primitifs est en correspondance biunivoque avec l'ensemble des suites d'entiers $(a_0, a_1, \ldots, a_k)$ (k entier ≥ 0 quelconque) telles que

$$a_0 = 0 \text{ si } \Pi = Z, \qquad a_0 = 0 \text{ ou } 1 \text{ si } \Pi = Z_{p^f}; \tag{1.2}$$

$$\left.\begin{aligned} &a_i \equiv 0 \text{ ou } 1 \pmod{2p-2} \text{ pour } 0 \leq i \leq k; \\ &a_1 \geq 2p-2, \qquad a_{i+1} \geq pa_i \qquad (1 \leq i \leq k-1). \end{aligned}\right\} \tag{1.3}$$

Le degré stable q et l'indice m sont donnés par

$$q = a_0 + a_1 + \ldots + a_k, \tag{1.4}$$

$$m + q = \left[\frac{p}{p-1}\, a_k\right] \tag{1.5}$$

(on note $[\lambda]$ le plus petit entier $> \lambda$). Mais la formule (1.5) est en défaut si $p = 2$ et a_k impair.

Or, pour $p = 2$, $E(m; Z_2) \otimes P(2m; Z_2) \approx P(m; Z_2)$; d'où une *construction d'algèbre initiale* $P(m; Z_2)$ *et d'algèbre finale* $\otimes_{k \geq 0} P(2^k m + 1; Z_2)$. La suspension envoie $x^{(2^k)} \in P(m; Z_2)$ dans le "générateur" de $P(2^k m + 1; Z_2)$. Par itération, l'algèbre d'homologie $H_*(\Pi, n; Z_2)$ est un produit tensoriel d'algèbres de polynômes modifiées; les générateurs primitifs admettent encore la description (1.2), (1.3), et les formules (1.4) et (1.5) sont valables sans restriction. D'où:

THÉORÈME 3.—*Pour $n \geq 1$, p premier impair, l'algèbre d'homologie $H_*(\Pi, n; Z_p)$ ($\Pi = Z$ ou Z_{p^f}) est isomorphe à un produit tensoriel d'algèbres extérieures (à un générateur de degré impair) et d'algèbres de polynômes modifiées (à un générateur de degré pair). Pour $n \geq 2$, $p = 2$, l'algèbre d'homologie $H_*(\Pi, n; Z_2)$ ($\Pi = Z$ ou Z_{2^f}) est isomorphe à un produit tensoriel d'algèbres de polynômes modifiées. Dans tous les cas, le nombre des générateurs dont le degré stable est q est celui des suites $(a_0, \ldots, a_k)$ satisfaisant aux formules* (1.2), (1.3), *et* (1.4), *et telles que $pa_k < (p-1)(n+q)$.*

2. *Les Algèbres de Cohomologie $H^*(\Pi, n; Z_p)$.*—Notons $P^*(m; \Lambda)$ l'algèbre des polynômes $\Lambda[u]$ à un générateur u de degré m, munie de la *multiplication ordinaire;* $E^*(m; \Lambda)$ désigne la même chose que $E(m; \Lambda)$.

On sait que l'algèbre de cohomologie $H^*(Z_{p^f}, 1; Z_p)$ est isomorphe à $E^*(1, Z_p) \otimes P^*(2; Z_p)$, sauf si $p^f = 2$; et que $H^*(Z_2, 1; Z_2) \approx P^*(1; Z_2)$. Dans chacune des constructions I et II ci-dessus, on peut définir un opérateur s qui en fait une construction *spéciale* (cf. I, § 3). En appliquant la méthode de I, § 7, on trouve, par itération:

THÉORÈME 4.[1]—*Pour $n \geq 1$, p premier impair, l'algèbre de cohomologie $H^*(\Pi, n; Z_p)$ ($\Pi = Z$ ou Z_{p^f}) est isomorphe à un produit tensoriel d'algèbres extérieures (à un générateur de degré impair) et d'algèbres de polynômes ordinaires (à un générateur de degré pair). Pour $n \geq 2$, $p = 2$, $H^*(\Pi, n; Z_2)$ ($\Pi = Z$ ou Z_{2^f}) est isomorphe à un*

produit tensoriel d'algèbres de polynômes ordinaires. Dans tous les cas, le nombre des générateurs dont le degré stable est q est celui des suites $(a_0, \ldots, a_k)$ *satisfaisant aux formules* (1.2), (1.3), *et* (1.4), *et telles que* $pa_k < (p-1)(n+q)$.

3. *Constructions à Coefficients Entiers.*—Soit m un entier pair ≥ 2. On note simplement $E(m-1)$ l'algèbre extérieure $E(m-1; Z)$, et $P(m)$ l'algèbre $P(m; Z)$. Pour tout entier h, soit $E_h(m-1)$ l'algèbre graduée $E(m-1) \otimes P(m)$ munie de la différentielle $dx = 0$, $dy^{(k)} = hxy^{(k-1)}$ (x, y: générateurs de $E(m-1)$, $P(m)$); c'est une DGA-algèbre, et x s'appelle le "générateur" de $E_h(m-1)$. On introduit aussi une DGA-algèbre $P_h(m)$ dont la définition complète est trop longue pour être explicitée ici; il suffit de savoir ceci: comme algèbre graduée, $P_h(m) = P(m) \otimes C$, où C est une algèbre graduée dont tous les éléments de base (sauf l'élément 1) sont de degé $> m$; l'injection $x \to x \otimes 1$ de $P(m)$ dans $P_h(m)$ identifie $P(m)$ à une sous-algèbre de $P_h(m)$ sur laquelle la différentielle de $P_h(m)$ est nulle, et définit donc un homomorphisme $P(m) \to H_*(P_h(m))$; en fait, ceci identifie $H_*(P_h(m))$ à un quotient de $P(m)$. D'une façon précise, soit u le "générateur" de $P(m)$, qu'on appelle aussi le "générateur" de $P_h(m)$; l'élément $u^{(i)} \in P(m)$ a pour image dans $H_*(P_h(m))$ un élément dont l'ordre est le quotient de ih par le produit des composantes p-primaires de ih (relatives à tous les p premiers ne divisant pas h).

Une méthode analogue à celle du § 1, mais plus compliquée, donne le résultat suivant:

THÉORÈME 5.—*Soit* $\Pi = Z$ (*resp.* Z_h *avec* $h = p^f$). *Pour n impair, l'algèbre* $H_*(\Pi, n; Z)$ *est isomorphe à l'algèbre d'homologie d'un produit tensoriel de DGA-algèbres*[2] $E(n) \otimes G(\Pi, n)$ (*resp.* $E_h(n) \otimes G(\Pi, n)$); *pour n pair,* $H_*(\Pi, n; Z)$ *est isomorphe à l'algèbre d'homologie d'un produit tensoriel* $P(n) \otimes G(\Pi, n)$ (*resp.* $P_h(n) \otimes G(\Pi, n)$). *Dans tous les cas,* $G(\Pi, n)$ *est un produit tensoriel (en général infini) de DGA-algèbres de la forme* $E_p(m-1)$ *et* $P_p(m)$ *pour tous les p premiers (resp. pour l'unique p premier divisant h). Le nombre des "générateurs" de* $G(\Pi, n)$ *relatifs à un p premier, et de degré stable* $q \geq 1$, *est celui des suites* $(a_0, \ldots, a_k)$ *satisfaisant aux formules* (1.2), (1.3), (1.4) *et*

$$a_k \equiv 0 \pmod{2p-2}, \tag{3.1}$$

et telles que $pa_k < (p-1)(n+q)$.

Les constructions à coefficients entiers permettent aussi de déterminer les *opérateurs de Bockstein* dans la cohomologie $H^*(\Pi, n; Z_p)$. Soit X un complexe de cochaînes (à coefficients entiers, sans torsion); soit $x \in X$, de degré q, tel que $dx = (-1)^{q+1}p^f x'$; x' est un cocyle dont l'image dans $H^{q+1}(X \otimes Z_p)$ est transformé, par l'opérateur $\beta(p^f)$, de l'image de x dans $H^q(X \otimes Z_p)$. L'opérateur $\beta(p)$ est défini sur $H^q(X \otimes Z_p)$ et à valeurs dans $H^{q+1}(X \otimes Z_p)$; $\beta(p^f)$ n'est défini que sur le noyau de $\beta(p^{f-1})$, et prend ses valeurs dans le conoyau de $\beta(p^{f-1})$. Les $\beta(p^f)$ *commutent avec la suspension.*

On démontre: soit u_0 l'unique générateur de degré n de $H^*(Z_{p^f}, n; Z_p)$ ("classe fondamentale"); alors $\beta(p^h) \cdot u_0 = 0$ pour $h < f$, et $\beta(p^f) \cdot u_0$ est l'unique générateur de degré $n+1$. Pour $\Pi = Z$ ou Z_{p^f}, soit u un générateur de $H^*(\Pi, n; Z_p)$, de schéma $(a_0, \ldots, a_k)$ avec $k \geq 1$, $a_k \equiv 0 \pmod{2p-2}$; alors[3] $\beta(p) \cdot u$ est le générateur de schéma $(a_0', \ldots, a_k')$, avec $a_i' = a_i$ pour $i < k$ et $a_k' = a_k + 1$.

4. *Relation avec les Opérations de Steenrod.*—Soit $a = (2p-2)\lambda + \epsilon$, λ entier, $\epsilon = 0$ ou 1. Pour tout espace topologique X, définissons St_p^a: $H^i(X, Z_p) \to H^{i+a}(X, Z_p)$ comme suit: si $\epsilon = 0$, St_p^a est l'opération $\mathcal{P}_p^\lambda$ de Steenrod;[4] si $\epsilon = 1$, St_p^a est le composé $\beta(p) \circ \mathcal{P}_p^\lambda$ (pour $p = 2$, on prend simplement $St_2^a = Sq^a$, carré de Steenrod). Soit I une suite $(a_0, \ldots, a_k)$ satisfaisant à la formule (1.3); on pose $St_p^I = St_p^{a_k} \circ \ldots \circ St_p^{a_1}$ si $a_0 = 0$; si $a_0 = 1$, on pose $St_p^{I,f} = St_p^{a_k} \circ \ldots \circ St_p^{a_1} \circ \beta(p^f)$.

Les générateurs qui interviennent dans le Théorème 4 peuvent être choisis de plusieurs manières. Les opérations de Steenrod permettent de fixer ce choix; leurs propriétés vis-à-vis des puissances p-ièmes et de la suspension conduisent en effet au:

THÉORÈME 6.[1]—*Soit* $\Pi = Z$ *(resp.* $\Pi = Z_{p^f}$*), et soit* u_0 *la classe fondamentale de* $H^*(\Pi, n; Z_p)$. *On peut prendre comme système de générateurs des algèbres extérieures et des algèbres de polynômes du Théorème 4, l'ensemble des* $St_p^I(u_0)$ *(resp. des* $St_p^I(u_0)$ *et des* $St_p^{I,f}(u_0)$*), où* I *parcourt l'ensemble des suites* $(a_0, \ldots, a_k)$ *satisfaisant aux formules* (1.2) *et* (1.3), *et telles que* $pa_k < (p-1)(n + a_0 + \ldots + a_k)$.

COROLLAIRE. Pour un entier q donné, l'ensemble des $St_p^I(u_0)$ (resp. des $St_p^I(u_0)$ et des $St_p^{I,f}(u_0)$), où I parcourt l'ensemble des suites $(a_0, \ldots, a_k)$ satisfaisant *aux* formules (1.2), (1.3), et (1.4), est une *base* du Z_p-espace vectoriel $H^{n+q}(\Pi, n; Z_p)$, dès que $n > q$.

5. *Calcul des "Groupes Stables"* $H_{n+q}(\Pi, n; Z)$, $n > q$.—Les opérations de Steenrod St_p^I sont transposées d'homomorphismes dans l'*homologie*, qu'on notera St_I^p. Soit Π un groupe abélien *quelconque;* si $I = (a_0, \ldots, a_k)$, avec $a_0 = 0$, $a_1 + \ldots + a_k = q$, soit θ_I^p l'homomorphisme composé

$$H_{n+q}(\Pi, n; Z) \to H_{n+q}(\Pi, n; Z_p) \xrightarrow{St_I^p} H_n(\Pi, n; Z_p) \approx \Pi/p\Pi.$$

Si $I = (a_0, \ldots, a_k)$, avec $a_0 = 1$, $a_1 + \ldots + a_k = q - 1$, soit θ_I^p le composé

$$H_{n+q}(\Pi, n; Z) \to H_{n+q}(\Pi, n; Z_p) \xrightarrow{St_J^p} H_{n+1}(\Pi, n; Z_p) \approx {}_p\Pi,$$

où J désigne $(a_1, \ldots, a_k)$, et ${}_p\Pi$ désigne le groupe des éléments d'ordre p de Π.

THÉORÈME 7.[5]—*Pour* $q \geq 1$, *et* $n > q$, $H_{n+q}(\Pi, n; Z)$ *est un groupe de torsion; soit* $L_q(\Pi; p)$ *sa composante p-primaire. Pour chaque suite* $I = (a_0, \ldots, a_k)$ *telle que* $a_0 = 0$ *ou* 1, *et satisfaisant aux formules* (1.3), (1.4), *et* (3.1), θ_I^p *applique* $L_q(\Pi; p)$ *sur* $\Pi/p\Pi$, *resp. sur* ${}_p\Pi$. *Soit* N_I^p *l'intersection des noyaux des* θ_J^p *pour toutes les suites* $J \neq I$; *alors* $L_q(\Pi; p)$ *est somme directe des* N_I^p, *et* θ_I^p *est un isomorphisme de* N_I^p *sur* $\Pi/p\Pi$, *resp. sur* ${}_p\Pi$.

COROLLAIRE. Pour $n > q \geq 1$, $H_{n+q}(\Pi, n; Z)$ est isomorphe (non canoniquement) à $H_{n+q}(Z, n; \Pi)$.

* Cette note fait suite à la précédente, citée "I." Voir, these PROCEEDINGS, **40**, 467–471, 1954.

[1] Ce théorème a déjà été démontré, dans le cas $p = 2$, par J. P. Serre, *Comm. Math. Helv.*, **27**, 198–231, 1953; voir §2.

[2] Le produit tensoriel de deux DGA-algèbres a été défini en I, §1.

[3] Il y a une exception: $p = 2$, $a_k + 1 = n + a_0 + \ldots + a_{k-1}$ impair; alors $\beta(2) \cdot u$ est le carré du générateur dont le schéma est $(a_0, \ldots, a_{k-1})$.

[4] N. Steenrod, these PROCEEDINGS, **39**, 217–223, 1953, formule (6.8).

[5] Les groupes stables $H_{n+q}(\Pi, n; Z)$ $(n > q)$, pour Π abélien quelconque, étaient connus au moins pour $q \leq 3$; dans le cas $\Pi = Z$, ils avaient été calculés pour $q \leq 10$ (Eilenberg-Mac Lane, these PROCEEDINGS, **36**, 657–663, 1950). On trouve aussi chez Serre, *op. cit.*, des renseignements sur la composante 2-primaire des groupes stables $H_{n+q}(\Pi, n; Z)$.

A NONLINEAR DIFFERENTIAL-DIFFERENCE EQUATION OF GROWTH

By W. J. Cunningham

YALE UNIVERSITY, NEW HAVEN, CONNECTICUT

Communicated by G. E. Hutchinson, June 20, 1954

The nonlinear differential-difference equation

$$\frac{dx(t)}{dt} = [a - bx(t - \tau)]x(t) \tag{1}$$

has been suggested[1] as a mathematical description of a fluctuating population of organisms under certain conditions. In this equation, a, b, and τ are positive real constants, while $x(t)$ and $x(t - \tau)$ are the populations at times (t) and $(t - \tau)$, respectively. The quantity in brackets represents the rate of growth at any instant, and it depends in part upon the population at an earlier instant in time.

The equation may apply to other than biological situations also. In particular, it might describe the operation of a control system working with a potentially explosive chemical reaction, provided a fixed time delay exists in the sensing element of the control. The equation also is quite similar to equations that arise in economic studies of the business cycle, where time delays exist in various steps of business operations.[2]

Methods of finding exact solutions for equations of this type seem to be unknown.[3] Qualitatively, it is evident that if $x(t)$ ever vanishes, the value of $dx(t)/dt$ also must vanish, and $x(t)$ can never become different from zero again. Thus, if $x(t)$ is not zero, it must always retain the same algebraic sign, and the signs of $x(t)$ and $x(t - \tau)$ must be identical. Only the case of $x(t)$ positive is considered here. If $x(t)$ is positive, the sign of $dx(t)/dt$ can be either positive or negative, depending upon the relative magnitudes of terms in equation (1). Thus oscillation is allowed with $x(t)$ positive. If $x(t)$ is negative, the sign of $dx(t)/dt$ is always negative, and the solution can only go to negative infinity.

When the combination of parameters is such that a periodic oscillation occurs, the mean value of x is a constant,

$$\bar{x} = \frac{a}{b}. \tag{2}$$

This result can be obtained by dividing both sides of equation (1) by $x(t)$, averaging over a period, and taking account of the periodicity.

A degenerate case of equation (1) occurs if delay time τ is zero, giving the Verhulst-Pearl equation.[4] It is an example of a Bernoulli equation and has the exact solution

$$x = \left[\frac{b}{a} + \left(x_0^{-1} - \frac{b}{a}\right) \exp\,(-at)\right]^{-1}, \tag{3}$$

where $x = x_0$ at $t = 0$.

An equation similar to equation (1), but simpler to analyze, results if the term

with the delay time is replaced by the Taylor's series

$$x(t - \tau) = x(t) - (\tau)\frac{dx(t)}{dt} + \left(\frac{\tau^2}{2}\right)\frac{d^2x(t)}{dt^2} \cdots \tag{4}$$

Provided that the delay time τ is sufficiently small and the higher-order derivatives are not too large, only the first few terms of this infinite series may represent it fairly accurately. Substitution of the first three terms gives

$$\ddot{x} - \alpha^2\tau\dot{x} + \frac{\alpha^2\dot{x}}{bx} + \alpha^2 x = \alpha^2\beta, \tag{5}$$

where $\alpha^2 \equiv 2/\tau^2$, $\beta \equiv a/b$. This second-order equation is nonlinear but has all terms evaluated at the same instant. In many cases of interest, its solutions should be similar to those of equation (1). Actually, equation (1) is equivalent to an infinite-order differential equation which might have an infinity of modes of oscillation, while equation (5) can have only a single such mode.

The third term of equation (5) is nonlinear and represents positive damping so long as x is positive. The second term represents negative damping. For certain combinations of the parameters, this nonlinear total damping might be expected to lead to an oscillatory solution having a limit cycle. Because the positive damping occurs asymmetrically during the cycle, the solution should have an asymmetric wave form. The well known van der Pol equation[5] leads to such a limit cycle, but there positive damping is symmetrical, as is the wave form.

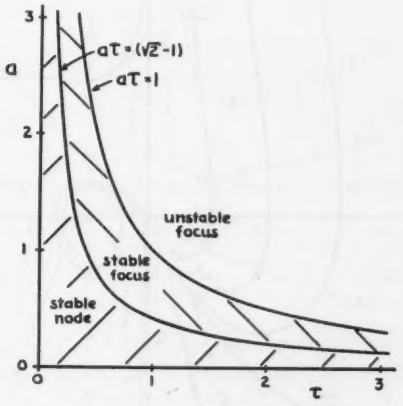


FIG. 1.—Stability diagram for solutions of eq. (5) near the singular point, $x_s = \beta$, $z_s = \tau\dot{x}_s = 0$.

The qualitative nature of solutions for equation (5) can be determined by studying the singular points[6] of the first-order equation resulting from the substitution $\tau\dot{x} = z$, so that $\tau^2\ddot{x} = z\,dz/dx$. This equation is

$$\frac{dz}{dx} = \frac{2[(1 - 1/b\tau x)z - x + \beta]}{z}. \tag{6}$$

Its solution can be represented on a phase plane having as axes the co-ordinates x and z. Singular points are located where both numerator and denominator of equation (6) vanish simultaneously. The only simple singularity, found this way, is located at $x_s = \beta$, $z_s = 0$. A more complicated singularity exists at the origin. The nature of the solution near the first singularity can be found by replacing x with $(x_s + u)$ and z with $(z_s + v)$, where u and v are small. If only linear terms are retained, increment u is given by

$$\ddot{u} - \alpha^2\left(\tau - \frac{1}{a}\right)\dot{u} + \alpha^2 u = 0, \tag{7}$$

which has characteristic roots

$$(\lambda_1, \lambda_2) = (a\tau^2)^{-1}\{(a\tau - 1) \pm [(a\tau - 1)^2 - 2a^2\tau^2]^{1/2}\}. \quad (8)$$

A stability diagram for solutions near the singular point (x_s, z_s) can be drawn as in Figure 1, making use of information in equation (8). The term "focus" describes an oscillatory solution; "node" describes a solution approaching a final value monotonically. An unstable solution has a positive value for the real part of at least one root.

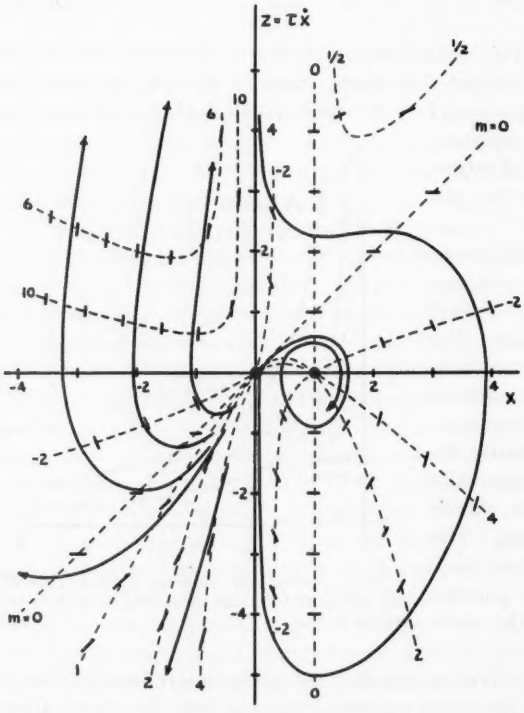


FIG. 2.—Isocline construction for solving eq. (5), with numerical values $\beta = 1$, $b\tau = 1$, $a\tau = 1$.

Solution curves for equation (5) can be found graphically by a construction based on equation (6), which can be considered to give the slope of a solution curve at any point in the phase plane. If this slope is assigned a constant value, say m, the equation for an isocline curve[7] is

$$z = b\tau x(\beta - x)\left[1 + b\tau x\left(\frac{m}{2} - 1\right)\right]^{-1}. \quad (9)$$

All isocline curves pass through the singular points (x_s, z_s) and the origin. At the origin the slope of all isoclines is the same, $dz/dx = a\tau$.

An example of such a construction is shown in Figure 2, where the isoclines are dotted and carry line segments with the appropriate slope. Several solution curves are sketched as solid lines, with time increasing in the direction of the arrows. The parameters for this figure lead to an oscillatory solution about (x_s, z_s), as indicated by the spiral curve converging toward this point. If x is negative, solution curves may go through large negative values of x but ultimately return to positive x, passing through infinite values of z on the way.

The singularity at the origin is evidently unstable in this example. For positive x, it is like a saddle point; for negative x, it is like an unstable nodal point.

Approximate solutions for equation (5), valid near (x_s, z_s), can be found analytically by two methods. In each case the generating solution

$$x = \beta + A \sin(\alpha t + \theta) \quad (10)$$

is used, where A is the amplitude and θ is the phase angle of the oscillatory solution. By applying the method of variation of parameters,[8] the amplitude is found to vary with time as

$$A = pA - qA^3, \tag{11}$$

where $p \equiv (a\tau - 1)(a\tau^2)^{-1}$ and $q \equiv b^2/4a^3\tau^2$. It is assumed here that $A/\beta < 1$ and that the relative change in A per cycle of oscillation is small. This last requirement is equivalent to the statement

$$4/5 < (a\tau) < 5/4.$$

Equation (11) has the solution

$$A = A(t) = \left[\frac{q}{p} + \left(A_0^{-2} - \frac{q}{p}\right)\exp(-2pt)\right]^{-1/2}, \tag{12}$$

where $A = A_0$ at $t = 0$. If p is positive, A always approaches the value

$$A_s = \left(\frac{p}{q}\right)^{1/2} = 2\beta(a\tau - 1)^{1/2}. \tag{13}$$

If p is negative, A approaches zero. In a similar way, it can be shown that on the average θ does not vary with time. Thus an approximate solution is

$$x = \beta + A(t)\sin\left[\left(\frac{2^{1/2}}{\tau}\right)t + \theta_0\right], \tag{14}$$

where $A(t)$ is given by equation (12) and A_0 and θ_0 depend upon initial conditions.

By applying a perturbation method,[9] another approximate solution is found to be

$$x = \beta + A\sin\alpha t - (\alpha A^2/6b\beta^2)\sin 2\alpha t \ldots \tag{15}$$

This is a steady-state solution valid for

$$1 < (a\tau) < 5/4.$$

In equation (15), A has the value of equation (13), and the presence of a second-harmonic component is indicated. Its phase is such as to reduce the rate of rise of x from small values and to steepen its drop from large values.

The approximate solutions, given in equations (14) and (15), apply directly to equation (5) and might be expected to apply reasonably well to equation (1), also. As mentioned earlier, equation (1) is equivalent to a differential equation of infinite order and might have an infinity of modes of oscillation. The mode with the longest period is found in the approximate solution of equation (15). This period is $T = 2^{1/2}\pi\tau$, and the ratio τ/T is in the order of $1/4$. Provided that the product $a\tau$ is large enough, modes with shorter periods may exist for which the ratio τ/T would be of the order of $5/4$, $9/4$, ... In any physical situation described by equation (1), it is likely that only the mode with longest period would be found.

An electronic analogue computer was used to find solutions for both equations

(1) and (5), with specific values for the numerical parameters. In Figure 3 are shown solutions for the pure differential equation, equation (5). The mean value

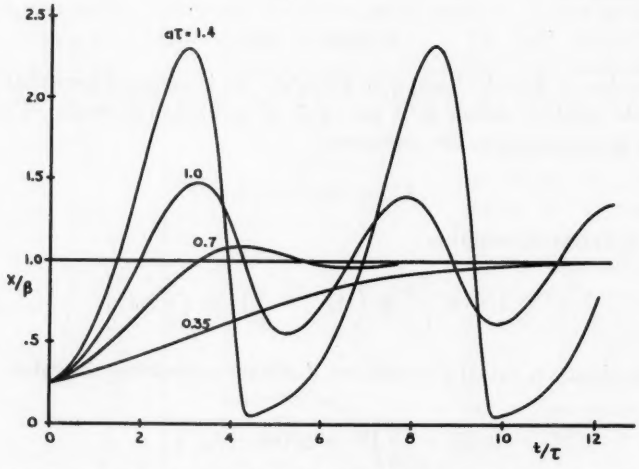


FIG. 3.—Solutions for eq. (5) found with analogue computer. At $t = 0$, $x_0 = \beta/4$, $\dot{x}_0 = 0$.

of the solution is β, and this value is approached monotonically if $a\tau < 0.4$, approximately. Sustained oscillations occur if $a\tau > 1.0$, approximately. As $a\tau$ is made larger, the oscillations become more violent in nature. Good agreement exists with the solutions found analytically. In Figure 4 are shown solutions for the differen-

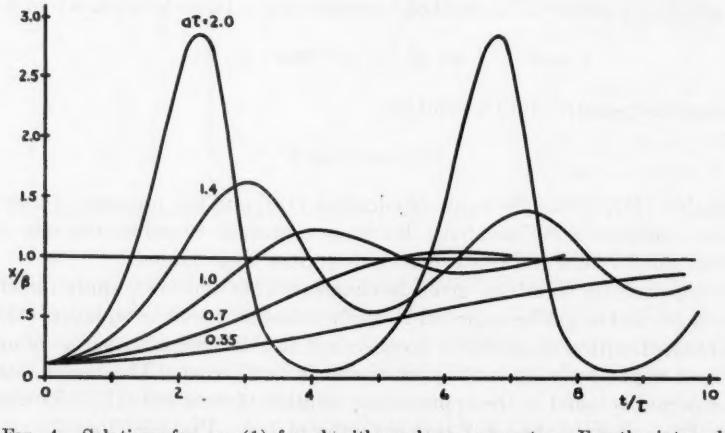


FIG. 4.—Solutions for eq. (1) found with analogue computer. For the interval $-\tau \leq t \leq 0$, $x = \beta/10$.

tial-difference equation, equation (1). Qualitatively they are similar to those of Figure 3, but a larger value of $a\tau$ is needed to give analogous curves. For example,

sustained oscillations do not occur unless $a\tau > 1.8$, approximately. As x rises from an initial small positive value, x_0, the curve of x versus t must fall between bounding curves given by equation (3) for $\tau = 0$ and by $x_0 \exp(at)$ for $\tau \to \infty$.

If product $a\tau$ is made very large, the solution becomes a relaxation oscillation about the mean value β, going to a large positive value, x_{max} and dropping suddenly very close to zero. A rather crude simplification of the wave form leads to rough estimates of x_{max} and the period T, for $a\tau$ very large:

$$x_{max} = (1 + a\tau)\beta, \tag{16}$$

$$T = \left(1 + \frac{a\tau}{2}\right)\tau. \tag{17}$$

The work described here was done in part under research contract Nonr-433(00) between the Office of Naval Research and Yale University.[10] The author is indebted to Professor G. E. Hutchinson for interesting discussions concerning the problem.

[1] G. E. Hutchinson, *Ann. N.Y. Acad. Sci.*, **50**, 221, 1948.

[2] R. M. Goodwin, *Econometrica*, **19**, 1, 1951.

[3] R. Bellman and J. M. Danskin, *Proceedings of the Symposium on Nonlinear Circuit Analysis* (New York: Polytechnic Institute of Brooklyn, 1953), p. 107. A long bibliography is included here.

[4] A. J. Lotka, *Elements of Physical Biology* (Baltimore: Williams & Wilkins Co., 1925), p. 64.

[5] N. Minorsky, *Nonlinear Mechancis* (Ann. Arbor, Mich.: J. W. Edwards, 1947), p. 113.

[6] *Ibid.*, chap. iii.

[7] *Ibid.*, p. 20.

[8] *Ibid.*, chap. x.

[9] *Ibid.*, chap. viii.

[10] W. J. Cunningham, "A Nonlinear Differential-Difference Equation of Growth" (Contract Nonr-433[00], Yale University, New Haven, May 1, 1954).

A NEW CLASS OF SIMPLE LIE ALGEBRAS

BY MARGUERITE STRAUS FRANK

UNIVERSITY OF CHICAGO

Communicated by A. A. Albert, April 30, 1954

1. *The Jacobson-Witt Algebra.*—In this note we shall define a class of Lie algebras $\mathfrak{T}_n$ of characteristic p. They will be subalgebras of the well-known Jacobson-Witt algebras. We shall prove that the algebras $\mathfrak{T}_n$ are simple for every $n > 2$ and that they have dimension $(n - 1)(p^n - 1)$. Since this is, in general, a new dimension, the algebras form a new class of simple Lie algebras of characteristic p.

Let $\mathfrak{F}$ be any field of characteristic p, $\mathfrak{B}_n = \mathfrak{F}[x_1, \ldots, x_n]$ be the algebra of all polynomials in $x_1, \ldots, x_n$ subject only to the condition that $x_1^p = \ldots = x_n^p = 0$. Then the dimension over $\mathfrak{F}$ of $\mathfrak{B}_n$ is p^n. It is easy to show that the algebra $\mathfrak{W}_n$ of all derivations over $\mathfrak{F}$ of $\mathfrak{B}_n$ is the set of all transformations

$$A: \quad f = f(x_1, \ldots, x_n) \to fA = \frac{\partial f}{\partial x_1} a_1 + \ldots + \frac{\partial f}{\partial x_n} a_n,$$

for $a_1, \ldots, a_n$ in $\mathfrak{B}_n$. Thus every derivation of $\mathfrak{B}_n$ may be represented by an n-tuple $A = (a_1, \ldots, a_n)$ with co-ordinates in $\mathfrak{B}_n$. It follows that the dimension of $\mathfrak{W}_n$ over $\mathfrak{F}$ is np^n.

The set $\mathfrak{W}_n$ is a Lie algebra over F with respect to the product $(f)\ (AB) = (fA)B - (fB)A$. It may be verified by an elementary computation that

$$(a_1, \ldots, a_n) \cdot (b_1, \ldots, b_n) = (c_1, \ldots, c_n), \tag{1}$$

where

$$c_i = \sum_{j=1}^{n} \left(\frac{\partial a_i}{\partial x_j} b_j - \frac{\partial b_i}{\partial x_j} a_j \right) \qquad (i = 1, \ldots, n). \tag{2}$$

In our study of certain subalgebras of $\mathfrak{W}_n$ it will be convenient to define certain special derivations. Write $D_i(d)$ for the derivation whose ith co-ordinate is d in $\mathfrak{B}_n$, and whose remaining co-ordinates are zero. We also write $D_{ij}(d) = (g_1, \ldots, g_n)$, where $g_k = 0$ for $k \neq i, j$, and

$$g_i = \frac{\partial d}{\partial x_j}, \ g_j = -\frac{\partial d}{\partial x_i} \qquad (i \neq j;\ i, j = 1, \ldots, n) \tag{3}$$

Evidently $D_{ij}(d)$ does not exist unless $n > 1$, and *we shall assume henceforth that* $n > 1$. Observe that

$$D_{ji}(d) = -D_{ij}(d). \tag{4}$$

We shall close this introductory section with a proof of the following computational tool result.

LEMMA 1. *Let* $A = (a_1, \ldots, a_n)$. *Then* $A \cdot D_i(d) = (c_1, \ldots, c_n)$, *where*

$$c_k = \frac{\partial a_k}{\partial x_i} d, \ c_i = \frac{\partial a_i}{\partial x_i} d - \sum_{j=1}^{n} \frac{\partial d}{\partial x_j} a_j \qquad (k \neq i;\ i, k = 1, \ldots, n). \tag{5}$$

If $n > 2$ *and* i, j, k *are distinct, then*

$$D_{ij}(d) \cdot D_{ik}(x_i x_k) = D_{ij}(h), \tag{6}$$

where

$$h = \frac{\partial d}{\partial x_i} x_i - \frac{\partial d}{\partial x_k} x_k - d. \tag{7}$$

Formula (5) is the special case of equation (2) in which $d = b_i$ and $b_k = 0$ for $k \neq i$. To derive the second result, we use equation (2) with $a_s = 0$ for $s \neq i, j$ and $a_i = \partial d/\partial x_j$, $-a_j = \partial d/\partial x_i$, $b_t = 0$ for $t \neq i, k$, $b_i = x_i$, $b_k = -x_k$. Then $D_{ij}(d) \cdot D_{ik}(x_i x_k) = (c_1, \ldots, c_n)$, where clearly $c_s = 0$ for $s \neq i, j, k$. But $a_k = 0$ and $\partial b_k/\partial x_j = 0$ for $j \neq k$, so that equation (2) implies that $c_k = 0$. We compute

$$c_i = \frac{\partial a_i}{\partial x_i} x_i - \frac{\partial a_i}{\partial x_k} x_k - a_i = \frac{\partial h}{\partial x_j} \tag{8}$$

by the fact that $a_i = \partial d/\partial x_j$. Also $a_j = -\partial d/\partial x_i$, and so

$$c_j = \frac{\partial a_j}{\partial x_i} x_i - \frac{\partial a_j}{\partial x_k} x_k = -\frac{\partial h}{\partial x_i}, \tag{9}$$

since

$$\frac{\partial}{\partial x_i}\left(\frac{\partial d}{\partial x_i} x_i\right) = \frac{\partial^2 d}{\partial x_i^2} x_i + \frac{\partial d}{\partial x_i}. \tag{10}$$

This completes our proof.

2. *Derivations Whose Divergence Is Zero.*—The *divergence* of any derivation $A = (a_1, \ldots, a_n)$ is defined to be the element

$$\delta(A) = \frac{\partial a_1}{\partial x_1} + \ldots + \frac{\partial a_n}{\partial x_n} \tag{11}$$

of $\mathfrak{B}_n$. We compute

$$\delta(A \cdot B) = \sum_{i=1}^{n} \frac{\partial c_i}{\partial x_i} = \sum_{j=1}^{n}\left[\frac{\partial}{\partial x_j}\left(\sum_{i=1}^{n} \frac{\partial a_i}{\partial x_i}\right)\right] b_j -$$
$$\sum_{j=1}^{n}\left[\frac{\partial}{\partial x_j}\left(\sum_{i=1}^{n} \frac{\partial b_i}{\partial x_i}\right)\right] a_j + \sum_{i,j=1}^{n}\left(\frac{\partial a_i}{\partial x_j}\right)\left(\frac{\partial b_j}{\partial x_i}\right) - \left(\frac{\partial a_j}{\partial x_i}\right)\left(\frac{\partial b_i}{\partial x_j}\right) =$$
$$\sum_{j=1}^{n} \frac{\partial}{\partial x_j}[\delta(A) b_j] - \sum_{j=1}^{n} \frac{\partial}{\partial x_j}[\delta(B) a_j]. \tag{12}$$

The result of formula (12) may be stated as follows, where we define $d(a_1, \ldots, a_n) = (da_1, \ldots, da_n)$ for every d of $\mathfrak{B}_n$.

LEMMA 2. *Let A and B be derivations. Then*

$$\delta(A \cdot B) = \delta[\delta(A)B] - \delta[\delta(B)A]. \tag{13}$$

We now derive our first major result.

THEOREM 1. *The set $\mathfrak{M}_n$ of all derivations A of divergence zero in a subalgebra of $\mathfrak{W}_n$. The dimension of $\mathfrak{M}_n$ over $\mathfrak{F}$ is $r_n = (n-1)p^n + 1$.*

For equation (13) implies that if $\delta(A) = 0$ and $\delta(B) = 0$, then $\delta(A \cdot B) = 0$. Since $\delta(A)$ is a linear function of A, we see that $\mathfrak{M}_n$ is a vector space over F. Hence $\mathfrak{M}_n$ is a subalgebra of $\mathfrak{W}_n$. Every A of $\mathfrak{M}_n$ has the property

$$-\frac{\partial a_n}{\partial x_n} = \frac{\partial a_1}{\partial x_1} + \ldots + \frac{\partial a_{n-1}}{\partial x_{n-1}}. \tag{14}$$

Write $x_n = x$ and

$$a_i = a_{i0} + a_{i1}x + \ldots + a_{i\,p-1}x^{p-1} \qquad (i = 1, \ldots, n), \tag{15}$$

where the a_{ij} are in $B_{n-1} = \mathfrak{F}[x_1, \ldots, x_{n-1}]$. If $n = 1$, the derivative of $a_1 = a = a(x)$ is zero, and so a is in $\mathfrak{F}$. Hence $r_1 = 1$, and the formula

$$r_n = (n-1)p^n + 1 \tag{16}$$

holds for $n = 1$. Assume, then, that $r_{n-1} = (n-2)p^{n-1} + 1$. We compute

$$\frac{\partial a_i}{\partial x_i} = \frac{\partial a_{i0}}{\partial x_i} + \frac{\partial a_{i1}}{\partial x_i} x + \ldots + \frac{\partial a_{i\ p-1}}{\partial x_i} x^{p-1} \qquad (i = 1, \ldots, n-1), \quad (17)$$

and

$$\frac{\partial a_n}{\partial x_n} = a_{n1} + 2a_{n2}x + \ldots + (p-1)a_{n\ p-1}\, x^{p-2}. \quad (18)$$

Thus equation (14) is equivalent to

$$ka_{nk} = -\sum_{i=1}^{n-1} \frac{\partial a_{i\ k-1}}{\partial x_i}, \sum_{i=1}^{n-1} \frac{\partial a_{i\ p-1}}{\partial x_i} = 0 \qquad (k = 1, \ldots, p-1). \quad (19)$$

It follows that the $(n-1)(p-1)$ vectors a_{ik} are arbitrary elements of $\mathfrak{B}_{n-1}$ for $i = 1, \ldots, n-1$ and $k = 0, \ldots, p-2$. Also, a_{n0} is an arbitrary element of $\mathfrak{B}_{n-1}$. The vectors a_{nk} are determined by equation (19) for $k = 1, \ldots, p-1$. The vector $(a_{1\ p-1}, a_{2\ p-1}, \ldots, a_{n-1\ p-1})$ is an arbitrary vector of $\mathfrak{M}_{n-1}$. It follows immediately that the dimension of $\mathfrak{M}_n$ is

$$(n-2)p^{n-1} + 1 + (p-1)(n-1)p^{n-1} + p^{n-1} = (n-1)p^n + 1,$$

as desired.

3. *Truncated Derivations.*—Every polynomial in $\mathfrak{B}_n$ may be expressed in its i-form,

$$a = \phi_{i0} + \phi_{i1} x_i + \ldots + \phi_{i\ p-1} x_i^{p-1}, \quad (20)$$

where the ϕ_{ij} are in $B_{n-1}^{(i)} = F[x_1, \ldots, x_{i-1}, x_{i+1}, \ldots, x_n]$. Then we shall say that a is *i-truncated* if the coefficient of

$$y_i = (x_1 x_2 \ldots x_{i-1} x_{i+1} \ldots x_n)^{p-1} \quad (21)$$

in every ϕ_{ij} of a is zero.

LEMMA 3. *An element a of $\mathfrak{B}_n$ is i-truncated if and only if there exist elements a_j in $\mathfrak{B}_n$ such that*

$$a = \sum_{j \neq i} \frac{\partial a_j}{\partial x_j}. \quad (22)$$

For if a is expressible in the form (22), it should be obvious that the term y_i never appears as a factor of a coefficient of any power of x_i, and so a is i-truncated. Conversely, if a is i-truncated, we may express a as the sum $a = \sum_{j \neq i} g_j$, where each g_j is of degree at most $p-2$ in a variable x_j. But then $g_j = \partial a_j/\partial x_j$ for an a_j in $\mathfrak{B}_n$. A derivation $A = (a_1, \ldots, a_n)$ is said to be *truncated* if $\delta(A) = 0$ and a_i is i-truncated. Since $\delta(A)$ is linear and all linear combinations over F of i-truncated polynomials are i-truncated, we see that *the set $\mathfrak{T}_n$ of all truncated derivations is a vector subspace of $\mathfrak{M}_n$.*

LEMMA 4. *The set $\mathfrak{T}_n$ is a subalgebra of $\mathfrak{M}_n$. Its dimension over $\mathfrak{F}$ is*

$$t_n = (n-1)(p^n - 1), \quad (23)$$

and it contains $\mathfrak{M}_n{}^2$.

For proof we compute

$$\sum_{i=1}^{n} \frac{\partial}{\partial x_j}(a_i b_j - b_i a_j) = \sum_{i=1}^{n}\left[\left(\frac{\partial a_i}{\partial x_j}\right) b_j\right] + a_i\left[\sum_{j=1}^{n}\left(\frac{\partial b_j}{\partial x_i}\right)\right] - \sum_{j=1}^{n}\left[\left(\frac{\partial b_i}{\partial x_j}\right) a_j\right] - \left[\sum_{j=1}^{n}\left(\frac{\partial a_j}{\partial x_j}\right)\right] b_i. \quad (24)$$

Then, if A and B are in $\mathfrak{M}_n$, we see that

$$A \cdot B = C, \quad (25)$$

where

$$c_i = \sum_{j \neq i} \frac{\partial}{\partial x_j}(a_i b_j - a_j b_i). \quad (26)$$

But then

$$A \cdot B = \sum_{i<j} D_{ij}(a_i b_j - a_j b_i). \quad (27)$$

By Lemma 3 we see that $A \cdot B$ is in $\mathfrak{T}_n$ for every A and B of $\mathfrak{M}_n$. Hence $\mathfrak{M}_n{}^2 \subseteq \mathfrak{T}_n$. But then $T_n{}^2 \subseteq \mathfrak{M}_n{}^2 \subseteq \mathfrak{T}_n$, and so $\mathfrak{T}_n$ is a subalgebra of $\mathfrak{M}_n$. By using equation (19) with n replaced by i, we see that the condition $\delta(A) = 0$ implies that

$$a_i = h_{i0} + h_{i1}x_i + \ldots + h_{i\,p-1}\, x_i^{p-1} \quad (28)$$

is i-truncated if and only if the polynomial h_{i0} is i-truncated. It follows that while the condition $\delta(A) = 0$ is satisfied for arbitrary a_{i0}, the condition that A be truncated reduces the dimension of the space of allowable a_{i0} by one. It follows that

$$t_n = r_n - n = (n-1)p^n + 1 - n = (n-1)\,(p^n - 1),$$

as desired. This completes our proof.

4. *A Second Characterization of* $\mathfrak{T}_n$.—Let $\mathfrak{M}_{ij} = \mathfrak{M}_{ji}$ be the subspace of $\mathfrak{M}_n$ spanned by the derivations $D_{ij}(a) = D_{ji}(-a)$ for all a in $\mathfrak{B}_n$, and let $\mathfrak{S}_n$ *be the sum of the* subspaces $\mathfrak{M}_{ij}$ of $\mathfrak{M}_n$. By equation (27) we see that

$$\mathfrak{M}_n{}^2 \subseteq S_n \subseteq \mathfrak{T}_n.$$

Then $\mathfrak{S}_n{}^2 \subseteq \mathfrak{S}_n$, and so $\mathfrak{S}_n$ is a subalgebra of $\mathfrak{T}_n$. We now prove a result which states that $\mathfrak{S}_n$ is actually a second version of $\mathfrak{T}_n$.

THEOREM 2. *The algebra* $\mathfrak{S}_n$ *is equal to* $\mathfrak{T}_n$.

For proof we define the *length* of a derivation $A = (a_1, \ldots, a_n)$ to be the number $\lambda(A)$ of nonzero co-ordinates a_i of A. If $\lambda(A) = 1$, and A is in $\mathfrak{T}_n$, we may assume that

$$A = D_1(a)$$

for a in $\mathfrak{B}_n$ without loss of generality. Then $\partial a/\partial x_1 = 0$, and so a is in $\mathfrak{B}_{n-1}{}^{(1)} = \mathfrak{F}[x_2, \ldots, x_n]$. Since A is truncated, we see that a is a sum of terms of the form $\phi_i x_i^j$, where $j < p-1$ and ϕ_i is in $\mathfrak{B}_{n-2}{}^{(i)} = \mathfrak{F}[x_2, \ldots, x_{i-1}, x_{i+1}, \ldots, x_n]$. But $\mathfrak{S}_n$ contains $(j+1)^{-1} D_{1i}(\phi_i x_i^{j+1}) = D_1(\phi_i x_i^j)$, and we have shown that $\mathfrak{S}_n$ contains every A of $\mathfrak{T}_n$ with $\lambda(A) = 1$.

Assume the inductive hypothesis that $\mathfrak{S}_n$ contains every A of $\mathfrak{T}_n$ with $\lambda(A) <$

t, and let $\lambda(A) = t$. There is no loss of generality if we assume that $A = (a_1, \ldots, a_t, 0, \ldots, 0)$. Write $x = x_t$ and

$$a_t = h_{t0} + h_{t1}\, x + \ldots + h_{t\, p-1}\, x^{p-1}, \tag{29}$$

where the h_{tj} are in $\mathfrak{B}_{n-1}^{(t)}$. We apply the property

$$-\frac{\partial a_t}{\partial x_t} = \frac{\partial a_1}{\partial x_1} + \ldots + \frac{\partial a_{t-1}}{\partial x_{t-1}} \tag{30}$$

as in equation (14), to obtain

$$k h_{tk} = -\sum_{i=1}^{t-1} \frac{\partial h_{i\, k-1}}{\partial x_t} \qquad (k = 1, \ldots, p-1). \tag{31}$$

But $D_{tt}(h_{i\, k-1}\, x^k)$ is a vector whose tth co-ordinate is

$$g_{tt} = -\frac{\partial h_{i\, k-1}}{\partial x_t}\, x^k$$

for $i = 1, \ldots, t-1$, and which is such that the co-ordinates $g_{tj} = 0$ for $j > t$. It follows that, if

$$C = A - \sum_{i=1}^{t-1} \sum_{k=1}^{p-1} k^{-1} D_{tt}(h_{i\, k-1} x^k), \tag{32}$$

then $C = (c_1, \ldots, c_t, 0, \ldots, 0)$, C is in $\mathfrak{S}_n$ if and only if A is in $\mathfrak{S}_n$, C is truncated, c_t is in $\mathfrak{B}_{n-1}^{(t)}$. Then c_t is t-truncated, and we may write

$$c_t = \sum_{j \neq t} \frac{\partial d_j}{\partial x_j}, \qquad \frac{\partial d_j}{\partial x_t} = 0. \tag{33}$$

We also know that $D_{jt}(d_j) = D_t(-(\partial d_j/\partial x_j))$, by the last equation of (33). But then $C + \sum_{j \neq t} D_{jt}(d_j) = E$, where $\lambda(E) < t$, E is in $\mathfrak{T}_n$. By our inductive hypothesis, E is in $\mathfrak{S}_n$, C is in $\mathfrak{S}_n$, A is in $\mathfrak{S}_n$, and so $\mathfrak{S}_n = \mathfrak{T}_n$.

5. *The Simplicity of* $\mathfrak{T}_n$.—The principal result of our theory may be stated as follows:

THEOREM 3. *Let* $n > 2$. *Then* $\mathfrak{T}_n$ *is a simple Lie algebra of dimension* $(n-1)(p^n - 1)$ *over its center* $\mathfrak{F}$ *and* $\mathfrak{T}_n = \mathfrak{M}_n^2$.

For let $\mathfrak{L}$ be a nonzero ideal of $\mathfrak{T}_n$ and A be any nonzero derivation in $\mathfrak{L}$. By Lemma 1 we see that $\mathfrak{L}$ contains $A_0 = A \cdot D_i(1) = (d_1, \ldots, d_n)$, where

$$d_j = \frac{\partial a_j}{\partial x_i} \qquad (j = 1, \ldots, n). \tag{34}$$

It follows that the result of partially differentiating all the co-ordinates of A with respect to x_i is in $\mathfrak{L}$ for every i. But then the successive partial differentiation of the co-ordinates of A will yield a derivation $G = (\gamma_1, \ldots, \gamma_n)$ in $\mathfrak{L}$, where the γ_i are all in $\mathfrak{F}$ and at least one $\gamma_k \neq 0$. We may actually assume that $\gamma_k = 1$ for some k. The vector $D_i(x_k)$ is in $\mathfrak{T}_n$ for every $i \neq k$, since $n > 2$. Then $\mathfrak{L}$ contains $G \cdot D_i(x_k) = -D_i(1)$ for every $i \neq k$. Also $\mathfrak{L}$ contains $D_k(x_i) \cdot D_i(1) = D_k(1)$. Hence L contains

$$D_i(1)$$

for $i = 1, \ldots, n$.

To see that $\mathfrak{L}$ contains $\mathfrak{T}_n = \mathfrak{S}_n$, we shall show that $\mathfrak{L}$ contains every $D_{ij}(a)$. By symmetry it suffices to show that $\mathfrak{L}$ contains $D_{12}(a)$. Since $\mathfrak{L}$, $\mathfrak{M}_{12}$ and $\mathfrak{B}_n$ are vector spaces, it is sufficient to show that $D_{12}(a)$ is in $\mathfrak{L}$ for every monomial

$$a = x_1^{i_1} \ldots x_n^{i_n} \qquad (i_k = 0, 1, \ldots, p-1). \tag{35}$$

Now $D_{12}(a)$ is in $\mathfrak{T}_n = \mathfrak{S}_n$, and so $\mathfrak{L}$ contains $D_{12}(a) \cdot D_k(1) = D_{12}(g)$ for $k = 1, \ldots, n$ by equations (5), where

$$g = \frac{\partial a}{\partial x_k}.$$

It follows that $\mathfrak{L}$ contains $D_{12}(a)$ for all monomials (35), except possibly for

$$a = z = (x_1 \ldots x_n)^{p-1}.$$

By Lemma 1, with $d = z$, we see that $\mathfrak{L}$ contains $-D_{12}(h)$, where

$$h = \frac{\partial z}{\partial x_1} x_1 - \frac{\partial z}{\partial x_3} x_3 - z = -z + z - z = -z.$$

Hence $\mathfrak{L}$ contains $D_{12}(z)$, and our proof that $\mathfrak{T}_n$ is simple is complete. The proof is independent of $\mathfrak{F}$ and implies that $\mathfrak{F}$ is the center of $\mathfrak{T}_n$. Evidently $\mathfrak{M}_n^2$ is a nonzero ideal of $\mathfrak{T}_n$, and so $\mathfrak{T}_n = \mathfrak{M}_n^2$.

The author wishes to acknowledge her debt to A. A. Albert for suggestions leading to the fairly simple proofs presented here.

---

*GENERAL EXISTENCE AND UNIQUENESS PROOF FOR SPATIALLY HOMOGENEOUS SOLUTIONS OF THE MAXWELL-BOLTZMANN EQUATION IN THE CASE OF MAXWELLIAN MOLECULES**

BY DIETRICH MORGENSTERN

GRADUATE INSTITUTE FOR APPLIED MATHEMATICS, INDIANA UNIVERSITY

Communicated by T. Y. Thomas, June 7, 1954

Existence of solutions of the initial value problem for the Maxwell-Boltzmann equation is an open problem. Recently Wild[1] has considered the integral equation

$$f(\xi, t) = F(\xi)\, e^{-\nu t} + \int_0^t e^{-\nu(t-\tau)} \nu \{f \circ f\}(\xi, \tau)\, d\tau, \tag{1}$$

where Wild's convolution $f \circ f$ is defined by

$$\nu F \circ G = \int_0^{2\pi} \int_0^{\pi} F(\mathfrak{n}) G(\mathfrak{n}') v \sigma(\theta, v)\, d\xi' \sin\theta\, d\theta\, d\varphi, \tag{2}$$

$\sigma(\theta, v)$ being the differential scattering cross-section and ν the mean collision frequency. When $v\sigma$ is independent of v, as is the case for Maxwellian molecules, then, setting the molecular mass equal to 1, we have

$$\nu = K\rho_0, \qquad K = \int_0^{2\pi} \int_0^{\pi} v\sigma(\theta, v) \sin\theta \, d\theta \, d\varphi. \tag{3}$$

Then, in so far as differentiable solutions are concerned, equation (1) is equivalent to the Maxwell-Boltzmann equation for a spatially homogeneous distribution $f(\xi, t)$ whose initial value is $F(\xi)$, the initial density being $\rho_0 = \int_\infty F(\xi) \, d\xi$. Wild formulated an iteration process for solving equation (1); however, he was able to prove its convergence only for a certain finite time interval and for $F(\xi)$ bounded by a Maxwellian distribution, and his proof is elaborate.

In this note, I show that Wild's iteration process converges for all t to an integrable solution of equation (1), provided only that the initial value satisfies the natural conditions $F(\xi) \geqq 0$ and $\int_\infty F(\xi) \, d\xi = \rho_0 < \infty$.

Wild's iterative scheme is

$$F_0(\xi, t) = F(\xi) e^{-\nu t}$$

$$F_{n+1}(\xi, t) = F_0(\xi, t) + \int_0^t e^{-\nu(t-\tau)} \nu\{F_n \circ F_n\}(\xi, \tau) \, d\tau. \tag{4}_1$$

Wild showed that $F_{n+1}(\xi, t) \geqq F_n(\xi, t)$. Introduce the densities of the iterates:

$$\rho_n(t) = \int_\infty F_n(\xi, t) \, d\xi.$$

These satisfy the recursion formula

$$\rho_{n+1}(t) = \rho_0(t) + \int_0^t e^{-\nu(t-\tau)} K[\rho_n(\tau)]^2 \, d\tau.$$

From equation $(4)_1$ it is plain that

$$\rho_0(t) = \rho_0 e^{-\nu t} \leqq \rho_0; \tag{4}_2$$

if $\rho_n(t) \leqq \rho_0$, from equation $(4)_2$ it follows that

$$\rho_{n+1}(t) \leqq \rho_0 e^{-\nu t} + \int_0^t e^{-\nu(t-\tau)} K\rho_0^2 \, d\tau = \rho_0.$$

Hence, by induction, $\rho_n(t) \leqq \rho_0$ for all n and all t. Therefore, by the theorem on integration of monotonic sequences with bounded integrals, the F_n converge for all t to an integrable limit function $F(\xi, t)$. This limit function obviously satisfies equation (1) and has the density $\rho(t) = \rho_0 = \text{const.}$

That this solution is the only spatially homogeneous one corresponding to $F(\xi)$ is easily proved. First, under more general circumstances Wild has shown that if g is a solution, then the iterative scheme starting from its initial value converges to a solution f such that $f \leqq g$. But if $f(\xi, t)$ and $g(\xi, t)$ are solutions having the same initial value, they necessarily have the same density. Hence

$$\int (g - f) \, \alpha\xi = \rho - \rho = 0.$$

Hence $g(\xi, t) = f(\xi, t)$ a.e. for each t. From the integral equation itself now follows

$$g \equiv f. \qquad \text{Q.E.D.}$$

Whether a nonhomogeneous solution $f(x, \xi, t)$ can develop from a homogeneous initial value $f(x, \xi, 0) = F(\xi)$ remains an open question.

In respect to the class of solutions and initial conditions, our existence theorem is the strongest yet obtained. The same arguments apply to the more general laws of molecular interaction defined by the functional equation

$$\int_\infty \int_0^{2\pi} \int_0^{\pi} v\sigma(\theta, v)\, F(\xi^1)\, d\xi^1 \sin\theta\, d\theta\, d\varphi = A \int_\infty F(\xi)\, d\xi + B \int_\infty |\xi|^2 F(\xi)\, d\xi$$

However, it does not seem possible to extend the proof so as to cover the general case. Thus the result of Carleman,[2] establishing in the case of perfectly elastic spherical molecules existence of solutions depending only on the speed $|\xi|$, is not included.

* This work, which arose in connection with Professor Truesdell's seminar on statistical mechanics, was done under joint contract of the Office of Naval Research and the Army Office of Ordnance Research with Indiana University.

[1] E. Wild, "On Boltzmann's Equation in the Kinetic Theory of Gases," *Proc Cambridge Phil. Soc.*, **47**, 602–609, 1951. Cf. also M. J. Klein, "A Note on Wild's Solution of the Boltzmann Equation," *Proc. Cambridge Phil. Soc.*, **50**, 239–297, 1954.

[2] T. Carleman, "Sur la solution de l'équation intégrodifférentielle de Boltzmann," *Acta math.*, **60**, 91–146, 1933.

ON THE ANALYSES OF $\{m\} \otimes \{1^k\}$ *AND* $\{m\} \otimes \{k\}$

By Francis D. Murnaghan

INSTITUTO TECNOLÓGICO DE AERONÁUTICA, SÃO JOSÉ DOS CAMPOS, BRASIL*

Communicated June 22, 1954

The representations $\{m\} \otimes \{1^k\}$ and $\{m\} \otimes \{k\}$ of the n-dimensional linear group, being constituents of the kth power of $\{m\}$, contain, when analyzed into their irreducible components, only those parentheses which have not more than k parts. We have given in a previous note[1] a simple method for determining those parentheses, in the analysis of $\{m\} \otimes \{1^k\}$ and $\{m\} \otimes \{k\}$, which contain the largest number, k, of parts: to determine the k-element parentheses of $\{m\} \otimes \{1^k\}$ add $\{1^k\}$ to each parenthesis of $\{m-1\} \otimes \{k\}$, and to determine the k-element parentheses of $\{m\} \otimes \{k\}$ add $\{1^k\}$ to each parenthesis of $\{m-1\} \otimes \{1^k\}$. We indicate these results as follows:

$$(\{m\} \otimes \{1^k\})' = \{m-1\} \otimes \{k\}; \quad (\{m\} \otimes \{k\})' = \{m-1\} \otimes \{1^k\},$$

where $(\{m\} \otimes \{1^k\})'$ denotes the result of subtracting $\{1^k\}$ from the k-element parentheses of $\{m\} \otimes \{1^k\}$ and $(\{m\} \otimes \{k\})'$ denotes the result of subtracting $\{1^k\}$ from the k-element parentheses of $\{m\} \otimes \{k\}$. Thus, since $\{2\} \otimes \{3\} = \{6\} + \{42\} + \{2^3\}$ and $\{2\} \otimes \{1^3\} = \{41^2\} + \{3^2\}$, the 3-element parentheses of $\{3\} \otimes \{1^3\}$ and $\{3\} \otimes \{3\}$ are $\{71^2\} + \{531\} + \{3^3\}$ and $\{52^2\} + \{4^21\}$, respectively. We complete, in the present note, this result by showing how to obtain the $(k-j)$-element parentheses in the analyses of $\{m\} \otimes \{1^k\}$ and $\{m\} \otimes \{k\}$, $j = 1, 2, \ldots, k-1$.

The formula which furnishes the $(k-j)$-element parentheses in the analysis of $\{m\} \otimes \{1^k\}$ may be written as follows:

$$(\{m\} \otimes \{1^k\})' = \{m\}(\{m\} \otimes \{1^{k-1}\})' - (\{m\} \otimes \{2\})(\{m\} \otimes \{1^{k-2}\})' + \ldots + (-1)^{j-1}(\{m\} \otimes \{j\})(\{m\} \otimes \{1^{k-j}\})' + (-1)^j(\{m-1\} \otimes \{k\})\{j\},$$

where the prime attached to a $(\ldots)$ denotes the result of subtracting $\{1^{k-j}\}$ from

each $(k - j)$-element parenthesis in $(\ldots)$, and all parentheses involving more than $k - j$ parts are neglected. Similarly,

$$(\{m\} \otimes \{k\})' = \{m\}(\{m\} \otimes \{k-1\})' - (\{m\} \otimes \{1^2\})(\{m\} \otimes \{k-2\})' + \ldots + (-1)^{j-1}(\{m\} \otimes \{1^j\})(\{m\} \otimes \{k-j\})' + (-1)^j(\{m-1\} \otimes \{1^k\})\{j\}.$$

For example, to obtain the 2-element parentheses in the analysis of $\{3\} \otimes \{1^3\}$, we set, with $j = 1$,

$$(\{3\} \otimes \{1^3\})' = \{3\}(\{3\} \otimes \{1^2\})' - (\{2\} \otimes \{3\})\{1\} = \{3\}(\{4\} + \{2^2\}) - (\{6\} + \{42\})\{1\} = (\{7\} + \{61\} + 2\{52\} + \{43\}) - (\{7\} + \{61\} + \{52\} + \{43\}) = \{52\}$$

and see that the desired result is $\{63\}$. $\{3\} \otimes \{1^3\}$ does not involve the 1-element partition $\{9\}$, and so the analysis of $\{3\} \otimes \{1^3\}$ is

$$\{3\} \otimes \{1^3\} = \{71^2\} + \{531\} + \{3^3\} + \{63\}.$$

Similarly, with $j = 1$,

$$(\{3\} \otimes \{3\})' = \{3\}(\{3\} \otimes \{2\})' - (\{2\} \otimes \{1^3\})\{1\} = \{3\}\{31\} - \{3^2\}\{1\} = (\{61\} + \{52\} + \{43\}) - \{43\} = \{61\} + \{52\},$$

so that the 2-element parentheses in the analysis of $\{3\} \otimes \{3\}$ are $\{72\} + \{63\}$. $\{3\} \otimes \{3\}$ involves the 1-element partition $\{9\}$ once, and so the analysis of $\{3\} \otimes \{3\}$ is

$$\{3\} \otimes \{3\} = \{52^2\} + \{4^21\} + \{72\} + \{63\} + \{9\}.$$

It will be sufficient, in order to illustrate the method, to consider the analyses of $\{3\} \times \{1^4\}$ and $\{3\} \otimes \{4\}$. Since $\{2\} \otimes \{4\} = \{8\} + \{62\} + \{4^2\} + \{42^2\} + \{2^4\}$ and $\{2\} \otimes \{1^4\} = \{51^3\} + \{431\}$, the 4-element parentheses in the analyses of $\{3\} \otimes \{1^4\}$ and $\{3\} \otimes \{4\}$ are $\{91^3\} + \{731^2\} + \{5^21^2\} + \{53^21\} + \{3^4\}$ and $\{62^3\} + \{5421\}$, respectively. To obtain the 3-element parentheses in $\{3\} \otimes \{1^4\}$, we set, with $j = 1$,

$$(\{3\} \otimes \{1^4\})' = \{3\}(\{3\} \otimes \{1^3\})' - (\{2\} \otimes \{4\})\{1\} = \{3\}(\{6\} + \{42\} + \{2^3\}) - (\{8\} + \{62\} + \{4^2\} + \{42^2\})\{1\} = (\{9\} + \{81\} + 2\{72\} + 2\{63\} + \{621\} + \{54\} + \{531\} + 2\{52^2\} + \{4^21\} + \{432\}) - (\{9\} + \{81\} + \{72\} + \{63\} + \{621\} + \{54\} + \{52^2\} + \{4^21\} + \{432\}) = \{72\} + \{63\} + \{531\} + \{52^2\},$$

so that the 3-element parentheses in the analysis of $\{3\} \otimes \{1^4\}$ are $\{831\} + \{741\} + \{642\} + \{63^2\}$. Similarly, we have, with $j = 1$,

$$(\{3\} \otimes \{4\})' = \{3\}(\{3\} \otimes \{3\})' - (\{2\} \otimes \{1^4\})\{1\} = \{3\}(\{41^2\} + \{3^2\}) - \{431\}\{1\} = (\{71^2\} + \{63\} + \{621\} + 2\{531\} + \{4^21\} + \{432\} + \{3^3\}) - (\{531\} + \{4^21\} + \{432\}) = \{71^2\} + \{63\} + \{621\} + \{531\} + \{3^3\},$$

so that the 3-element parentheses in the analysis of $\{3\} \otimes \{4\}$ are $\{82^2\} + \{741\} + \{732\} + \{642\} + \{4^3\}$. To obtain the 2-element parentheses in the analysis of $\{3\} \otimes \{1^4\}$, we set, with $j = 2$,

$$(\{3\} \otimes \{1^4\})' = \{3\}(\{3\} \otimes \{1^3\})' - (\{3\} \otimes \{2\})(\{3\} \otimes \{1^2\})' + (\{2\} \otimes \{4\})\{2\} = \{3\}\{52\} - (\{6\} + \{42\})(\{4\} + \{2^2\}) + (\{8\} + \{62\} + \{4^2\})\{2\} = (\{82\} + \{73\} + \{64\} + \{5^2\}) - (\{10\} + \{91\} + 3\{82\} + 2\{73\} + 3\{64\}) + (\{10\} + \{91\} + 2\{82\} + \{73\} + 2\{64\}) = \{5^2\},$$

so that the only 2-element parenthesis in the analysis of $\{3\} \otimes \{1^4\}$ is $\{6^2\}$. $\{3\} \otimes \{1^4\}$ does not involve the 1-element partition $\{12\}$, and so the analysis of $\{3\} \otimes \{1^4\}$ is

$$\{3\} \otimes \{1^4\} = \{91^3\} + \{731^2\} + \{5^21^2\} + \{53^21\} + \{3^4\} + \{831\} + \{741\} + \{642\} + \{63^2\} + \{6^2\}.$$

Similarly, to obtain the 2-element parentheses in the analysis of $\{3\} \otimes \{4\}$, we set, with $j = 2$,

$$(\{3\} \otimes \{4\})' = \{3\}(\{3\} \otimes \{3\})' - (\{3\} \otimes \{1^2\})(\{3\} \otimes \{2\})' + (\{2\} \otimes \{1^4\})\{2\} = \{3\}(\{61\} + \{52\}) - (\{51\} + \{3^2\})\{31\} = (\{91\} + 2\{82\} + 2\{73\} + 2\{64\} + \{5^2\}) - (\{82\} + \{73\} + 2\{64\}) = \{91\} + \{82\} + \{73\} + \{5^2\},$$

so that the 2-element parentheses in the analysis of $\{3\} \otimes \{4\}$ are $\{10.2\} + \{93\} + \{84\} + \{6^2\}$. $\{3\} \otimes \{4\}$ contains the 1-element parenthesis $\{12\}$ once, and so the analysis of $\{3\} \otimes \{4\}$ is

$$\{3\} \otimes \{4\} = \{62^3\} + \{5421\} + \{82^2\} + \{741\} + \{732\} + \{642\} + \{4^3\} + \{10.2\} + \{93\} + \{84\} + \{6^2\} + \{12\}.$$

* Present address: 6202 Sycamore Road, Baltimore 12, Md.

[1] These PROCEEDINGS, **37**, 439–441, 1951.

THE REDUCTION OF THE INNER PRODUCT OF TWO IRREDUCIBLE REPRESENTATIONS OF S_n

BY G. DE B. ROBINSON AND O. E. TAULBEE

DEPARTMENT OF MATHEMATICS, MICHIGAN STATE COLLEGE

Communicated by Oscar Zariski, June 14, 1954

1. *Introduction.*—If (α) and (β), of degrees f_α and f_β, are two irreducible representations of a finite group G of order g, then the reduction of the *inner product*[1] (Kronecker product[2]) $(\alpha) \times (\beta)$ of degree $f_\alpha f_\beta$ is of general interest. If H is a subgroup of G of order h, let us denote the representation of G *induced* by the representation (γ) of H by $(\gamma)H \uparrow G$ of degree gf_γ/h. Similarly, let us denote the representation of H obtained by *restricting* the representation (β) of G by $(\beta)G \downarrow H$. We denote the identity representation by (I). The following theorem must have been noted many times:

THEOREM 1.

$$((I)H \uparrow G) \times (\beta) = ((\beta)G \downarrow H) \uparrow G. \tag{1}$$

The proof is immediate, since the character on each side of equation (1) is

$$\frac{gn_R{}^H}{hn_R}\chi_R{}^{(\beta)}$$

where n_R is the number of conjugates of R in G, $n_R{}^H$ of these lying in H.

The significance of Theorem 1 becomes apparent if we take $G = S_n$ and utilize our special knowledge of the symmetric group as summarized in i–v below.

(i) Every irreducible representation $[\alpha]$ of S_n corresponds to a partition of n and so to a Young diagram containing α_1 nodes in the first row, α_2 nodes in the second, and so on, where $\alpha_1 \geq \alpha_2 \geq \ldots \geq \alpha_k$ and $\alpha_1 + \alpha_2 + \ldots + \alpha_k = n$.

(ii) Thus with each irreducible representation $[\alpha]$ is associated a subgroup[4, 5]

$$H_\alpha = S_{\alpha_1} \times S_{\alpha_2} \times \ldots \times S_{\alpha_k},$$

which is the direct product of the symmetric groups corresponding to the rows of $[\alpha]$.

(iii) Moreover, the irreducible components of the permutation representation $(I)H_\alpha \uparrow S_n$ are given by the Young *operator*,[3] which we shall make explicit in the following section.

(iv) For such a subgroup H_α, the representation $([\beta]S_n \downarrow H_\alpha) \uparrow S_n$ appearing on the right-hand side of equation (1) is a sum of *skew* representations or *outer products*[1, 4] whose irreducible components are obtainable by applying the Littlewood-Richardson rule.[3]

(v) Finally, interchanging rows and columns of $[\alpha]$ yields the *associated* representations $[\alpha']$. Since the characters of even classes are the same and those of odd classes are opposite in sign in $[\alpha]$ and $[\alpha']$, we conclude that, if

$$[\alpha] \times [\beta] = \sum c_{\alpha\beta\gamma}[\gamma],$$

then

$$c_{\alpha\beta\gamma} = c_{\beta\alpha\gamma} = c_{\alpha'\beta'\gamma} = c_{\beta'\alpha'\gamma}. \tag{2}$$

Thus, in constructing a table of inner products, it is not necessary to consider all possible combinations of factors.

2. *The Young Operator.*—The reduction of $(I)H_\alpha \uparrow S_n$ depends on the successive raising[3] of the nodes of $[\alpha]$ from the jth row to the ith row, denoted by the operator $\mathfrak{R}_{ij}$, for $i < j$. The resulting term is to be ignored (a) if the number of symbols in any row is less than the number in a subsequent row or (b) if two nodes from the same row appear in the same column.

Noting the analogy with the partition generating function, we write

$$\Pi(\mathfrak{R}) = \prod_{i<j} (1 - \mathfrak{R}_{ij})^{-1}, \tag{3}$$

$$\Pi(\mathfrak{R})^{-1} = \prod_{i<j} (1 - \mathfrak{R}_{ij}), \tag{4}$$

where $j = 2, 3, \ldots, k$. The following theorem shows the significance of these operators.

THEOREM 2. *The reduction of the permutation representation of S_n induced by the identity representation of H is given by*

$$(I)H_\alpha \uparrow S_n = \Pi(\mathfrak{R})[\alpha]. \tag{5}$$

This equation may be inverted to yield

$$[\alpha] = \Pi(\mathfrak{R})^{-1}((I)H_\alpha \uparrow S_n). \tag{6}$$

It is to be noted that $\Pi(\Re)^{-1}$ operates in equation (6) on a representation of S_n given by the skew diagram $[\alpha_1]\cdot[\alpha_2]\ldots[\alpha_k]$, whose constituents are single rows.[4] Since these rows are *disjoint*, restrictions a and b do not apply. The only requirement that a term contribute is that the excess of the number of appearances of j as a second subscript over the number of its appearances as a first subscript, in that term, shall not exceed α_j, for all j. Since the identity representation is contained once in each permutation representation, we conclude that the number of positive terms on the right of equation (6) is equal to the number of negative terms, if $k > 1$. The following example illustrates the theorem.

$$(I)H_{2,1,2} \uparrow S_4 = \Pi(\Re)\,[2, 1^2] = (1 - \Re_{12})^{-1}(1 - \Re_{23})^{-1}(1 - \Re_{13})^{-1}\,[2, 1^2]$$
$$= [2, 1^2] + [2^2] + 2[3, 1] + [4],$$

$$[2, 1^2] = \Pi(\Re)^{-1}\{(I)H_{2,1,2} \uparrow S_4\} = (I)H_{2,1,2} \uparrow S_4 - (I)H_{2,2} \uparrow S_4$$
$$- (I)H_{3,1} \uparrow S_4 + (I)H_4 \uparrow S_4. \quad (7)$$

3. *The Reduction of the Inner Product* $[\alpha] \times [\beta]$. Corresponding to equation (6), we may obviously write

$$[\alpha] \times [\beta] = \Pi(\Re)^{-1}((I)H_\alpha \uparrow S_n) \times [\beta]. \quad (8)$$

But, by Theorem 1 and iv, we know the reduction of each term appearing on the right-hand side of equation (8). We summarize the necessary steps in the following:

CONSTRUCTION. *To obtain the reduction of the inner product* $[\alpha] \times [\beta]$, *apply the operator* $\Pi(\Re)^{-1}$ *as in* (8), *then restrict the representation* $[\beta]$ *to the appropriate subgroup* H_α *of* S_n. *Each term thus obtained can be represented*[4] *as a sum of outer products whose irreducible components are obtainable by applying the Littlewood-Richardson rule.*[3]

The special case of this construction where $[\alpha] = [n - 1, 1]$ was given recently by Gamba and Radicati.[1] As is apparent, the complications increase rapidly, but we give the details of the method in the case of $[2, 1^2] \times [3, 1]$.

$$((I)H_4 \uparrow S_4) \times [3, 1] = [3, 1]$$
$$((I)H_{3,1} \uparrow S_4) \times [3, 1] = [3]\cdot[1] + [2, 1]\cdot[1] = [4] + 2[3, 1] + [2^2] + [2, 1^2]$$
$$((I)H_{2,2} \uparrow S_4) \times [3, 1] = [2]\cdot[1]\cdot[1] + [1^2]\cdot[2]$$
$$= [4] + 3[3, 1] + [2^2] + 2[2, 1^2]$$
$$((I)H_{2,1,1} \uparrow S_4) \times [3, 1] = 2[2]\cdot[1]\cdot[1] + [1^2]\cdot[1]\cdot[1]$$
$$= 2[4] + 5[3, 1] + 3[2^2] + 4[2, 1^2] + [1^4]$$

so that, finally, from equation (7):

$$[2, 1^2] \times [3, 1] = [3, 1] + [2^2] + [2, 1^2] + [1^4].$$

This result may be verified by reversing the order of the factors and applying the simpler construction appropriate to that case.[1]

[1] A. Gamba and L. A. Radicati, "Sopra un teorema per la riduzione di talune rapprasentazioni del gruppa simmetrico," *Atti accad. nazl. Lincei, Rend. Classe sci. fis. mat. e nat.*, **14**, 632–634, 1953.

[2] F. D. Murnaghan, "The Analysis of the Kronecker Product of Irreducible Representations of the Symmetric Group," *Am. J. Math.*, **60**, 761–784, 1938.

[3] G. de B. Robinson, "On the Representations of the Symmetric Group," *Am. J. Math.*, **60**, 745–760, 1938.

[4] G. de B. Robinson, "On the Representations of the Symmetric Group," (third Paper), *ibid.*, **70**, 277–295, 1948.

[5] R. M. Thrall and G. de B. Robinson, "Supplement to a Paper by G. de B. Robinson," *Am. J. Math.*, **73**, 721–724, 1951.

---

*ON A CLASS OF SEMISIMPLE RESTRICTED LIE ALGEBRAS**

By George B. Seligman†

NEW HAVEN, CONNECTICUT

Communicated by Nathan Jacobson, July 9, 1954

The structure of semisimple Lie algebras over fields of characteristic zero reduces to the determination of all simple Lie algebras. In case the base field is algebraically closed, this problem has been solved by Killing[1] and in more rigorous fashion by Cartan.[2] Except in the case of the three exceptional algebras of higher dimension, the problem for arbitrary fields of characteristic zero has been solved in terms of the results for algebraically closed fields.[3]

Beginning with papers of Jacobson and Zassenhaus,[4] considerable interest has arisen in Lie algebras whose base fields are of prime characteristic. Here the general structure of semisimple algebras is not known to any satisfactory degree. This is true even when the algebra is required to be *restricted* in the sense of Jacobson, i.e., to be closed under an operation $x \to x^{[p]}$ which coincides with the ordinary pth power in Lie subalgebras of associative algebras over fields of prime characteristic p. The results previously obtained along these lines consist mainly in the demonstration of classes of simple algebras, including some which have no analogues of characteristic zero.

A recent paper of Dieudonné[5] has shown that if a Lie algebra L has nondegenerate Killing form $Tr(\mathrm{ad}(x)\mathrm{ad}(y))$, then L is a direct sum of simple nonabelian ideals. Each of these ideals in turn has nondegenerate Killing form. Moreover, the work of Zassenhaus mentioned above proves that every derivation of L is inner. Now if $x \in L$, $(\mathrm{ad}(x))^p$ is a derivation of L, where p is the characteristic of the base field Φ. Thus $(\mathrm{ad}(x))^p = \mathrm{ad}(x^{[p]})$ for some (uniquely determined) element $x^{[p]} \in L$. Under the mapping $x \to x^{[p]}$, L is a restricted Lie algebra. By the definition of $x^{[p]}$, $\mathrm{ad}(x^{[p]}) = (\mathrm{ad}(x))^p$, i.e., the mapping $x \to \mathrm{ad}(x)$ is a *restricted representation* of L.

More generally, let L be a restricted Lie algebra containing no abelian ideals, and let $x \to U(x)$ be a restricted representation of L such that the form $Tr(U(x)U(y))$ is nondegenerate on L. It follows from the results of Dieudonné that L is a direct sum of simple nonabelian restricted ideals, on each of which the restriction of the form $Tr(U(x)U(y))$ is nondegenerate. Then the structure of L will be obtained by determining these simple factors, as in the case of the structure of semisimple algebras of characteristic zero.

In case the base field Φ is algebraically closed, the simple Lie algebras satisfying the above conditions (i.e., that they be restricted and possess a restricted repre-

sentation with nondegenerate trace form) have now been determined by the author. A detailed account of these results will appear in a forthcoming paper. Earlier work by Jacobson[6] enables one to extend these results in large part to arbitrary fields. The purpose of the present note is to describe in final form the main results of the author. In order to do this, it is useful to recall the following known classes of simple Lie algebras of characteristic p ($p > 2$):

A. All n by n matrices of trace zero over the field Φ of prime characteristic p, p not a divisor of n. If $p \mid n$, this algebra A_{n-1} has a one-dimensional center ΦI, and $PA_{n-1} = A_{n-1}/\Phi I$ is a simple restricted Lie algebra.

B. The algebras B_n of all $(2n + 1)$ by $(2n + 1)$ skew-symmetric Φ-matrices.

C. The algebras C_n of all $2n$ by $2n$ Φ-matrices M satisfying $S^{-1}M'S = -M$, where M' is the transpose of M, and

$$S = \left(\begin{array}{c|c} 0 & I_n \\ \hline -I_n & 0 \end{array}\right).$$

D. The algebras D_n of all $2n$ by $2n$ skew-symmetric Φ-matrices ($n \geq 4$).

E. For sufficiently large p, the analogues of the five exceptional simple algebras of characteristic zero.[7]

With the exception of $PA_{n-1}(p \mid n)$, one can easily find restricted representations of these algebras with nondegenerate trace forms, provided that the characteristic p is sufficiently large. The answer to this question for PA_{n-1} is unknown to the author; its Killing form is degenerate. The above is by no means a complete listing of the simple restricted Lie algebras. For example, the *Witt algebras* as characterized by Zassenhaus[8] and generalized by Jacobson[9] are simple.

Over an algebraically closed field, the existence of a nondegenerate restricted trace form enables one to prove that the decomposition of a simple algebra by a Cartan subalgebra satisfies many of the same conditions as in the case of characteristic zero. In particular, Cartan subalgebras are commutative, and the other root-spaces are one-dimensional. All roots are linear combinations with coefficients in the prime field of a set of roots linearly independent over Φ, and for $p > 7$ one can choose this linearly independent subset in such a manner that a certain associated matrix of integers (mod p) very nearly determines the algebra up to restricted isomorphism. Moreover, all possible matrices can be completely determined. Using these results, one can establish the following theorem:

THEOREM 1. *Let L be a simple restricted Lie algebra over an algebraically closed field Φ of characteristic $p > 7$. Suppose that L has a restricted representation with nondegenerate trace form. Then L is isomorphic to an algebra of one of the classes A, B, C, D, E.*

The answer to the converse question is not completely known. In addition to the uncertainty regarding PA_{n-1}, it is unknown whether the five exceptional algebras have nondegenerate trace forms for all $p > 7$. They are obtained from their analogues of characteristic zero by utilizing the fact that the latter have integral multiplication tables relative to suitable bases. The determinant of the Killing form is then an integer. If q is the highest prime factor in any of these five determinants, then the exceptional algebras of characteristic p have nondegenerate Killing forms if $p > q$. Combining these remarks with what is known regarding the Killing forms of the classes A–D, one has the following result:

THEOREM 2. *The simple Lie algebras with nondegenerate Killing form over an algebraically closed field of characteristic $p > max\ (q, 7)$ are the following:*

$$\begin{aligned} &A_n(p \nmid (n+1)); \\ &B_n(p \nmid (2n-1)), \quad n \geq 2; \\ &C_n(p \nmid (n+1)), \quad n \geq 3; \\ &D_n(p \nmid (n-1)), \quad n \geq 4; \end{aligned}$$

the analogues G_2, F_4, E_6, E_7, E_8 of the five exceptional simple Lie algebras of characteristic zero.

* The work here summarized was done in the Graduate School of Yale University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

† National Science Foundation Predoctoral Fellow.

[1] W. Killing, *Math. Ann.*, **31**, 252–290, 1888; **33**, 1–48, 1889.

[2] É. Cartan, thesis, Paris, 1894.

[3] N. Jacobson, *Duke Math. J.*, **4**, 534–551, 1938; **5**, 775–783, 1939; M. L. Tomber, *Proc. Am. Math. Soc.*, **4**, 759–768, 1953.

[4] N. Jacobson, *Trans. Am. Math. Soc.*, **42**, 206–224, 1937; H. Zassenhaus, *Abhandl. Math. Sem. Hamburg Univ.*, **13**, 1–100, 1939.

[5] J. Dieudonné, *Proc. Am. Math. Soc.*, **4**, 931–932, 1953.

[6] N. Jacobson, *Am. J. Math.*, **63**, 481–515, 1941.

[7] Professor Jacobson informs me that these have been investigated in unpublished work of Professor C. Chevalley.

[8] H. Zassenhaus, *op. cit.*, pp. 37–49.

[9] N. Jacobson, *Duke Math. J.*, **10**, 107–121, 1943.

HAMARTIEXÉRESIS AS APPLIED TO TABLES INVOLVING LOGARITHMS

BY HORACE S. UHLER

YALE UNIVERSITY

Communicated by E. Hille, June 26, 1954

One special case was presented in a paper[1] by this author which gave the results of omnibus checking of certain tables of 61-place denary logarithms. The basic idea was to compare, over a chosen interval ($k + 1$ to n) of the table to be tested, the value of log ($n!$) as computed from the sum log ($k!$) + log ($k + 1$) + log ($k + 2$) $+ \cdots +$ log ($n - 1$) + log n with the value of log ($n!$) as obtained from unimpeachable data and method. Any difference between these two evaluations of log ($n!$) would be ascribable to one or more errors in the table under examination. The point to be emphasized here is the fact that the evidence afforded by the given table of logarithms is contributed primarily through the sum of its logarithms.

In an analogous manner the logarithm of the product of a sequence of consecutive factorials equals the sum of the logarithms of the discrete factorials. Hence a table of log ($n!$) can be subpoenaed to give its evidence of taint of error or of absolute infallibility through the sum of its entries. The number of significant figures representing a fairly large factorial when enumerated on the base ten is enormous and practically useless. For illustration, 1000000! covers 5565709 places, which are

resolvable into 249998 terminal zeros and 5315711 initial significant figures. [Duarte's log $(n!)$, p. 128.] When, however, factorials are expressed as products of powers of prime factors, the testing calculations can be performed without difficulty.

The primary practical object of the present paper is to subject to hamartiexéresis the first thousand entries of a book by F.-J. Duarte bearing the title NOUVELLES TABLES DE LOG $n!$ À 33 DÉCIMALES DEPUIS $n = 1$ JUSQU'À $n = 3000$. Apparently those closely associated with Professor Duarte consider the main body of his tables to be absolutely free from error. In the Preface Professor R. de Montessus de Ballore says: "Ces Tables sont le résultat d'un grand effort et nous devons nous féliciter d'avoir en mains un instrument de calcul précieux, que la conscience et l'habileté de son auteur permet d'utiliser en toute sécurité." In a later book by Duarte having the title *Nouvelles tables logarithmiques a 36 décimales* we find a short list of "Errata des Nouvelles Tables de Log $n!$" on the unnumbered back of page xxvii. "Les quelques fautes d'impression que nous avons relevées n'altèrent pas l'exactitude de ces tables." These errors are here omitted as unimportant. We have noticed three more "innocent" misprints in the Introduction.

Finally, on March 26, 1954, the writer received the following letter from his collaborating erudite friend Professor D. H. Lehmer. We quote: "In reply to your letter of March 21, I know of no list of errors in F. J. Duarte, Nouvelles Tables de Log $n\cdots$. Geneva and Paris 1927, nor do I know of a single error. This table has not been reviewed in MTAC because it is not 'recent.' "

Since no significant errors have been reported in Duarte's table of log $(n!)$, we shall proceed to compare the sum of the first 1000 entries (pp. 3 through 42) with the value derived from $\prod_{1}^{1000}(n!)$. By desk machine the author found $\log[\prod_{1}^{1000}(n!)] =$ 117724.51130 94706 10844 59067 28120 64574 463$\cdots$. This datum was checked over and over again in various ways and with great care.

Table 1 gives the exponents e_n of all the primes in $\prod_{n=2}^{997}(n^{e_n})$, that is, in $2^{495562}\cdot 3^{247263}\cdot 5^{123124}\cdot \cdots \cdot 991^{10}\cdot 997^{4}$. In the opinion of the writer his procedure for the calculation of the exponents (e_n's) is important and perfectly general. It depends only upon the intrinsic definition of the term "factorial" and the values of the prime numbers within the interval under investigation. Nevertheless, in behalf of clarity the explanation will be based upon the special case of Duarte's table of log $(n!)$. Consider first the exponent 40508 of 13 in Table 1. The concept "factorial" indicates that 13 will maintain the same exponent throughout consecutive intervals of 13 places each. The first 12 factors $1\cdot 2\cdot 3\cdot\cdots\cdot 11\cdot 12$ can contribute nothing to the total exponent of 13. At $n = 13$ the exponent 1 persists as far as $n = 25$. From $n = 26$ to $n = 38$ the exponent of 13 will be 2. From $n = 39$ to $n = 51$ the exponent will be 3. This increase by one unit of exponent for every 13 places continues only as far as the bracket $n = 156$ to $n = 168$, exponent 12. At $n = 169$ the exponent rises 2 units because 169 is the square of 13. That is, we now have the exponent 14 from $n = 169$ to $n = 181$; 15 from $n = 182$ to $n = 194$, etc. At the cube of 13, i.e., 2197, the increase in the exponent would be 3. However, we cannot have $6 \times 169 = 1014$ because the limit 1000 was arbitrarily set at the start. Consequently the sum of the exponents for the factor 13 from $n = 1$ to $n = 1000$ equals $(13)[(1 + 2 + 3 + \cdots + 12) + (14 + 15 + \cdots + 26) + (28 + 29 + \cdots + 40)$

$+ (42 + 43 + \cdots + 54) + (56 + 57 + \cdots + 68) + (70 + 71 + \cdots + 81) = (13)[6\cdot13 + 20\cdot13 + 34\cdot13 + 48\cdot13 + 62\cdot13 + 6\cdot151] = (13)[170\cdot13 + 906] = 40508$. This result has been checked by slavishly adding the exponents in Alliaume's table of $n!$ as decomposed into prime factors from $n = 1$ to $n = 1200$. For $n > 1200$ the author's manuscript table to $n = 3400$ may be the only reliable table to this extent in existence. If we had chosen the factor 5 as an illustration, "jumps"

TABLE 1

n	e_n	n	e_n	n	e_n	n	e_n
2	495562	191	2140	439	685	709	292
3	247263	193	2110	443	673	719	282
5	123124	197	2050	449	655	727	274
7	81774	199	2020	457	631	733	268
11	48697	211	1894	461	619	739	262
13	40508	223	1774	463	613	743	258
17	30240	227	1734	467	601	751	250
19	26789	229	1714	479	565	757	244
23	21757	233	1674	487	541	761	240
29	16939	239	1614	491	529	769	232
31	15704	241	1594	499	505	773	228
37	13041	251	1497	503	498	787	214
41	11724	257	1461	509	492	797	204
43	11155	263	1425	521	480	809	192
47	10164	269	1389	523	478	811	190
53	8955	271	1377	541	460	821	180
59	7992	277	1341	547	454	823	178
61	7720	281	1317	557	444	827	174
67	6979	283	1305	563	438	829	172
71	6559	293	1245	569	432	839	162
73	6370	307	1161	571	430	853	148
79	5850	311	1137	577	424	857	144
83	5538	313	1125	587	414	859	142
89	5137	317	1101	593	408	863	138
97	4675	331	1017	599	402	877	124
101	4464	337	991	601	400	881	120
103	4374	347	961	607	394	883	118
107	4194	349	955	613	388	887	114
109	4104	353	943	617	384	907	94
113	3940	359	925	619	382	911	90
127	3451	367	901	631	370	919	82
131	3339	373	883	641	360	929	72
137	3171	379	865	643	358	937	64
139	3115	383	853	647	354	941	60
149	2877	389	835	653	348	947	54
151	2835	397	811	659	342	953	48
157	2709	401	799	661	340	967	34
163	2583	409	775	673	328	971	30
167	2500	419	745	677	324	977	24
173	2410	421	739	683	318	983	18
179	2320	431	709	691	310	991	10
181	2290	433	703	701	300	997	4

of 2, 3, 4, and 5 units would have occurred at $n = 25$, 125, 625, and 3125, respectively. For the imposed upper limit 1000 the $\sqrt{1000} = 31.6227766$ prevents the square of any prime greater than 31 from introducing a jump in the exponent. Of course Legendre's formula[2] $E_p(n!) = (n - \sum_{i=0}^{s} a_i)/(p - 1)$ was used as a check throughout the work.

Returning to the main problem, there was complete agreement as far as the 25th decimal place between the two approximations to $\log \prod_{1}^{1000}(n!)$ as calculated from

Duarte's Table 1 and from the writer's method. On the other hand, the sequences beginning with the 24th place read 20645 74463 and 19745 73558 for Duarte and Uhler, respectively. The difference 00900 0090(5) presumably indicated an error of 9 units in excess in each of the 26th and 31st places somewhere in Duarte's Table 1. The first error falls on page 6 in Duarte's log (99!). The seventh quartet reads $8\bar{9}29$ instead of $8\bar{0}29$. This is inexcusable because the mantissas of log (99!) and of log (100!) must be identical. The given value for log (100!) is correct.

The second alleged mistake could be accounted for by assuming an error of transposition ("Banker's error") on page 13 in Duarte's log (266!). The eighth quartet should be changed from $1\bar{9}87$ as printed to $1\bar{8}97$. To be very sure, we calculated *de novo* log (266!) from the 27th to the 33rd decimal place. The sum came out as 60 $1\bar{8}97$ 6166. The factorization of 266! was $2^{263}\cdot3^{130}\cdot5^{65}\cdot7^{43}\cdot11^{26}\cdot13^{21}\cdot17^{15}\cdot19^{14}\cdot23^{11}\cdot29^{9}\cdot31^{8}\cdot37^{7}\cdot41^{6}\cdot43^{6}\cdot47^{5}\cdot53^{5}\cdot59^{4}\cdot61^{4}\cdot67^{3}\cdot71^{3}\cdot73^{3}\cdot79^{3}\cdot83^{3}\cdot89^{2}\cdot97^{2}\cdot101^{2}\cdot103^{2}\cdot107^{2}\cdot109^{2}\cdot113^{2}\cdot127^{2}\cdot131^{2}\cdot137\cdot139\cdot149\cdot151\cdot157\cdot163\cdot167\cdot173\cdot179\cdot181\cdot191\cdot193\cdot197\cdot199\cdot211\cdot223\cdot227\cdot229\cdot233\cdot239\cdot241\cdot251\cdot257\cdot263$.

Too much emphasis cannot be placed upon the circumstance that regardless of whether no error is or many errors are detected by the method of hamartiexéresis, the absolute assurance is given that no noncanceling errors in the given interval have escaped detection.

In behalf of completeness it may not be without interest to record the following data. The product of all factorials from 1 to 1000 possesses 123124 terminal zeros. The total number of digits expressing $\prod_{1}^{1000}(n!)$ on the denary scale equals 1177246, hence the number of initial significant figures is 1054122. The very first extreme digits are 3 24570 81842 23678 30345 41022 15485 718 as calculated from the mantissa 0.51130 94706 10844 59067 28120 64574 463···.

During the short interval of time required by Dr. Wrench to examine the original manuscript of the preceding material, for possible improvement in style and accuracy of statement, the author extended the operation of hamartiexéresis to $n = 1200$, i.e., to the upper limit chosen by Duarte in his 1928–1929 book. The writer found that the sum of the data on pages 43–50 of Duarte's table of log $n!$ equals 5 74381. 32769 80836 22570 05738 05658 26823155.

Since the exponents 219114, 109340, ··· of 2, 3, ··· have no general applicability, it would be altogether wasteful to tabulate their values in this place. The likewise omitted additional primes are available in easily accessible reliable collections.

When the operations $219114 \log 2 + 109340 \log 3 + \cdots + 14 \log 1187 + 8 \log 1193$ were carried out with great care, the sum was found to agree absolutely with the sum of the values of log $(n!)$ as given earlier. The valuable conclusion is that no noncanceling errors exist in Duarte's table for $n = 1001$ to $n = 1200$.

For the benefit of the reader who may be interested in applying the above method to Duarte's table from $n = 1200$ to $n = 2000$ we furnish, from the sums of pages 51–82, the result that $\log\left[\prod_{1201}^{2000}(n!)\right]$ equals 35 54083. 06020 22365 38534 89693 34254 26090359.

[1] H. S. Uhler, "Omnibus Checking of the 61-Place Table of Denary Logarithms Compiled by Peters and Stein, by Callet, and by Parkhurst," these PROCEEDINGS, **39**, No. 6, 533–537, 1953.

[2] Harriet Griffin, "Elementary Theory of Numbers," (New York: McGraw-Hill Book Co., Inc., 1954), pp. 40–43.

EXAMINATION OF METHODS OF ATTACK ON THE SECOND CASE OF FERMAT'S LAST THEOREM

By H. S. Vandiver

UNIVERSITY OF TEXAS

Communicated June 7, 1954

1. *Introduction.*—In another paper[1] in these Proceedings, D. H. and Emma Lehmer and the writer proved that

$$x^n + y^n = z^n \tag{1}$$

has no nonzero solutions in rational integers for $2 < n < 2003$. This was accomplished by using criteria developed by Kummer (1850) and a modified form of a criterion due to the writer (1929) and testing these for each prime exponent in the range just mentioned, using the SWAC (the high-speed digital computing machine of the National Bureau of Standards at the Institute of Numerical Analysis at Los Angeles). In the present paper we shall show how this program was extended to $n < 2521$. The work on the SWAC was carried out by John Selfridge. Also, we shall discuss in detail how one might proceed in case the criteria so far employed in this program when applied to still larger exponents should fail in some particular case. This involves the consideration and comparison of various criteria for the second case of equation (1) which have been given by Inkeri and the writer.

2. *The Impossibility of Equation* (1) *for* $2000 < n < 2521$.—In Paper F we considered the equation

$$x^l + y^l + z^l = 0, \tag{2}$$

with l an odd prime. As is known, in order to prove equation (1) impossible for $n > 2$, it is sufficient to prove equation (2) impossible. The criteria employed[2] are, first, Kummer's result (Theorem I below) that equation (2) is impossible if l is a regular prime and, second, the following (cf. Paper F, Theorem III, p. 30), which is a transformation of Theorem II below.

Theorem A. *Let*

$$B_{a_1}, B_{a_2}, \ldots, B_{a_s} \tag{3}$$

be the only Bernoulli numbers, with indices $<(l-1)/2$ *which are divisible by* l. *Then, if*

$$Q_{a_i}{}^k \not\equiv 1 \pmod{p}$$

holds for $i = 1, 2, \ldots, s$, *Fermat's Last Theorem holds for the exponent* l.

The above Bernoulli numbers B_s are expressed in their lowest terms, and

$$B_1 = 1/6, \qquad B_2 = 1/30, \text{ etc.},$$

$$Q_a = t^{-kd/2} \prod_{b=1}^{\mu} (t^{kb} - 1)^{b^{l-1-2a}},$$

with $\mu = (l-1)/2$; p is a prime $<l^2 - l$ of the form $1 + kl$; t is any integer such that $t^k \not\equiv 1 \pmod{p}$; and

$$d = 1^{l-2a} + 2^{l-2a} + \ldots + \mu^{l-2a}.$$

Below is a table giving the values of l, $2a$, p, Q_a, and $Q_a{}^k$ modulo p, for all irregular l's; $2000 < l < 2521$. Here a is any of the a's which appear in expression (3). If a prime in the range mentioned does not appear in the table, it is regular, and Theorem I, below, applies to equation (2).

l	$2a$	p	Q_a	$Q_a{}^k$	l	$2a$	p	Q_a	$Q_a{}^k$
2003	60	4007	964	3679	2293	2040	9173	2893	6619
2003	600	4007	2510	1096	2309	1660	32327	25921	29869
2017	1204	8069	1171	5120	2309	1772	32327	27391	16798
2039	1300	4079	1625	1512	2357	2204	14143	1242	13699
2053	1932	94439	87471	29705	2371	242	52163	35658	12387
2087	376	37567	26723	23821	2371	2274	52163	37499	36924
2087	1298	37567	18226	3533	2377	1226	42787	13306	14718
2099	1230	25189	9760	5458	2381	2060	28573	23230	629
2111	1038	16889	15837	4962	2383	842	9533	2023	2339
2137	1624	12823	4875	445	2383	2278	9533	7774	3803
2143	1916	8573	3936	6283	2389	776	28669	10238	8820
2153	1832	12919	8367	6442	2411	2126	19289	3317	2048
2213	154	26557	22272	8338	2423	290	29077	12721	13088
2239	1826	22391	14707	488	2423	884	29077	17024	8817
2267	2234	45341	12723	41732	2441	366	48821	47645	47446
2273	876	4547	1035	2680	2441	1750	48821	46703	21851
2273	2166	4547	2351	2596	2503	1044	25031	13542	6421

It will be seen from the table that there are 39 regular and 26 irregular primes with $2000 < l < 2521$. This makes 223 regular and 144 irregular primes in the range $2 < l < 2521$, so that the percentage of irregular primes is running about 40 per cent of the total number of primes in those intervals. On further examination of the present table, we note also that there is no prime which has the rank of irregularity >2.

3. *Examination of Methods Concerning the Second Case of Equation* (1).— We shall presently state six theorems giving criteria for Fermat's Last Theorem. Theorem I, when combined with any one of the others, has yielded a proof of equation (2) for any particular l to which they have been applied. It will be noted that in the statements of these no separation is made of cases I and II of equation (2). This is because in the assumptions involving the E's (defined below) the statement that the second factor of the class number of the field K defined by $e^{2i\pi/l}$ is prime to l was included, and it was shown by the writer[3] that equation (2) was then impossible in case I. Hence we shall consider in further discussions here only case II of equation (2).

Let $k_i = \zeta^{r^i/2} - \zeta^{-r^i/2}$, $\zeta = e^{2i\pi/l}$, and $e_i = k_{i+1}/k_i$, with r a primitive root of l. Set

$$E_n = \prod_{i=0}^{\mu-1} e_i{}^{r^{-2in}}. \tag{4}$$

If $\mathfrak{p}$ is a prime ideal in K, then there is a natural number t such that $\alpha^P \equiv \zeta^t \pmod{\mathfrak{p}}$; $P = (N(\mathfrak{p}) - 1)/l$, with $N(\mathfrak{p})$ the norm of $\mathfrak{p}$, α in K, $((\alpha), \mathfrak{p}) = 1$ and $1 - \zeta = \lambda$, $\mathfrak{p} \neq (\lambda)$. Set

$$\zeta^t = \left(\frac{\alpha}{\mathfrak{p}}\right).$$

We now introduce some terminology used by Inkeri.[4] If

$$\left(\frac{E_a}{\mathfrak{p}_a}\right) \neq 1 \tag{5}$$

for any a in expression (3), then the set of prime ideals, distinct from (λ),

$$\mathfrak{p}_{a_1}, \mathfrak{p}_{a_2}, \ldots, \mathfrak{p}_{a_s}, \tag{6}$$

are said to form an E-set. In particular, if all these $\mathfrak{p}_a$'s are equal, say to $\mathfrak{p}$, then $\mathfrak{p}$ *is said to determine an E-set.* Kummer[5] (1850) proved:

THEOREM I. *If l is a given regular prime, then*

$$x^l + y^l + z^l = 0$$

is impossible for rational integers x, y, and z, none zero.

Then Vandiver[3] (1929 and 1934) found:

THEOREM II. *If there exists a prime ideal determining an E-set in K where p is a rational prime with $(p) \equiv 0 \pmod{\mathfrak{p}}$ and $p < l^2 - l$, $p = 1 + kl$, then equation* (1) *is impossible.*

THEOREM III. *If $p \equiv 1 \pmod{l}$ but $\not\equiv 1 \pmod{l^2}$ and $\mathfrak{p}$ is a prime ideal in K determining an E-set with $p \equiv 0 \pmod{\mathfrak{p}}$ and if the congruence*

$$u^l + v^l + w^l \equiv 0 \pmod{p} \tag{6a}$$

has no solutions in rational integers u, v, and w each $\not\equiv 0 \pmod{p}$, then equation (1) *is impossible.*

In 1949 Inkeri[6] obtained a generalization of Theorem II:

THEOREM IV. *If there is an E-set in K and if every rational prime which is divisible by a prime ideal of this set satisfies the conditions*

$$p < \frac{3(l^2 - l)}{2}, \qquad p \equiv 1 \pmod{l},$$

then equation (1) *is impossible.*

He also obtained, at the same time, the following generalization of Theorem III:

THEOREM V. *If there exists in K an E-set such that every rational prime p which is divisible by a prime ideal in this set is such that $p \equiv 1 \pmod{l}$ and $p \not\equiv 1 \pmod{l^2}$ and also the congruence* (6a) *has no solutions in u, v, and w each $\not\equiv 0 \pmod{p}$, then equation* (1) *is impossible.*

We also have:[3]

THEOREM VI. *Under the assumptions that* (1) *the second factor of the class number of the field K is prime to l;* (2) *none of the Bernoulli numbers B_{nl}, $n = 1, 2, \ldots, (l-3)/2$, is divisible by l^3, then equation* (1) *is impossible in nonzero integers x, y, and z.*

We shall now consider how to proceed in case some combination of Theorem I and one other breaks down for some particular l when examined later by the SWAC or some other computing machine. The first step in applying any of the criteria is to determine if l is regular. This procedure cannot fail because of Emma Lehmer's formula (cf. Paper F, formula (7)). In the first place, any such combination will fail if the second factor h_2 of the class number is divisible by l,

since, if it is, at least one of the E's in relation (5) is an lth power of an integer in K. This would vitiate criteria II-VI, inclusive, and also Theorem I, since l was assumed regular in the latter, whereas the assumption concerning h_2 would make K irregular. On the other hand, if it were proved that h_2 was prime to l for every l, this statement in itself would not necessarily enable us to prove equation (2) impossible for a particular l. Although the E's would not be lth powers, they would not necessarily form an E-set within the limits set for norms of the $\mathfrak{p}$'s, in Theorems II-V inclusive. In Theorem VI there is an assumption concerning the Bernoulli numbers which must also hold. The testing of Theorem I and Theorem II in Paper F, as described in the first part of the present paper, shows that none of the l's in expression (3) are lth powers in K for any $l < 2521$, so that $(h_2, l) = 1$ for those l's.

If, in testing Theorem II, we find a $\mathfrak{p}$ not satisfying the given conditions with $p = N(\mathfrak{p})$ (norm of $\mathfrak{p}$) in the range $l^2 - l$, then we have recourse to Theorem IV in order to try to find an E-set $\mathfrak{p}_1, \mathfrak{p}_2, \ldots, \mathfrak{p}_c$ with $N(\mathfrak{p}_i) < \frac{3(l^2 - l)}{2}$, $i = 1, 2, \ldots, c$. If this fails, then we may use Theorem V and test p's in the congruence (6a). This may be carried out on a machine of the SWAC type. A limit was found by A. Hurwitz[7] in terms of p beyond which relation (6a) always has solutions. After these tests, we may examine the p's referred to in relation (6a) and see if we can set up an E-set. If all the above-mentioned methods fail, and meanwhile however, someone proves that $(h_2, l) = 1$ for every l, then we may examine the second criteria in Theorem VI, using the congruence

$$(-1)^a \frac{B_{al}(2^{al} - 1)}{2^{2al}al} \equiv 1^{2al-1} + 2^{2al-1} + \ldots + \left(\frac{l-1}{2}\right)^{2al-1} \pmod{l^2}.$$

This gives $B_{al} \not\equiv 0 \pmod{l^3}$ for any $l < 293$. There is an excellent check on these calculations, as, since $B_a \equiv 0 \pmod{l}$, then B_{al} must be $\equiv 0 \pmod{l^2}$.

Theorem VI is a direct generalization of a criterion given by Kummer (1857) that equation (2) be satisfied when l is irregular. This is not the case for Theorems II–V, inclusive. (Cf. the discussion in the first reference in n. 2, pp. 574–575.)

[1] These PROCEEDINGS, **40**, 25–33, 1954; this article will be referred to as "Paper F."

[2] Any terms employed in this article which are not defined here are explained in H. S. Vandiver, *Am. Math. Monthly*, **53**, 569–571, 1946, or in Paper F.

[3] *Trans. Am. Math. Soc.*, **31**, 621, 631, 638, 1929. The proof for case I was given in *Bull. Am. Math. Soc.*, **40**, 118, 1934.

[4] *Ann. Acad. Sci. Fennicae Ser. A* (*I. Mathematica-Physica*), **60**, 4, 1949.

[5] *J. f. Math.*, **40**, 93–138, 1850; *J. de Math.*, **16**, 454–498, 1851.

[6] *Op. cit.*, pp. 18, 21.

[7] *J. f. Math.*, **136**, 272–279, 1909.

THE AGGLUTINATING AND SENSITIZING CAPACITY OF ANTISERA TO SHEEP RED CELLS AFTER VARYING DEGREES OF PHOTO-OXIDATION

By Albert Tyler,* M. Lorraine Fiset,† and Robin R. A. Coombs

California Institute of Technology, Pasadena, California, and Department of Pathology, University of Cambridge, Cambridge, England

Communicated by Linus Pauling, June 10, 1954

Tyler[1] showed that photo-oxidation could cause antisera to become nonprecipitating and nonagglutinating without destroying the ability of the antibodies to combine with the specific antigen. This was shown by the fact that treated antiserum could inhibit specifically agglutination or precipitation by corresponding untreated serum. On the basis of the mutual multivalence theory of Marrack,[2] Heidelberger,[3] and Pauling[4] it was considered that multivalent antibody had been made functionally univalent by the treatment, the term "univalent antibody" being used to designate an antibody that is incapable of causing precipitation or agglutination but which is still able to combine with the antigen.

A method more sensitive and direct than that of specific inhibition for demonstrating the presence of specifically combining but nonagglutinating antibody is the antiglobulin sensitization test of Coombs, Mourant, and Race.[5] However, this method was not available when Tyler's original experiments were performed. The present experiments show that it can be used with photo-oxidized antisera, and thus one can measure more directly the sensitizing capacity of sera rendered nonagglutinating by this method. There was also a more urgent reason for undertaking the present experiments on the photo-oxidation of rabbit anti–sheep red cell sera. Coombs, Howard, and Mynors[6] reported a serological procedure theoretically capable of detecting incomplete or nonprecipitating antibodies to soluble protein antigens. The method using ox red cells, although simple in theory, is rather lengthy to perform. A much shorter method, employing sheep red cells, was envisaged if a nonagglutinating rabbit sheep cell antibody were available.[7] Tyler's work showed the possibility of producing this type of antibody by photo-oxidation. Chemically linked to such an antibody, a soluble protein antigen, such as egg albumin, could be fixed to the surface of sheep cells, which would nevertheless remain stable in suspension. This cell system could then be used for the specific absorption of incomplete antibody, in human serum, to the conjugated protein—the absorption being detected finally by an agglutination reaction using a rabbit anti–human globulin serum. Preliminary studies in this direction have been promising, and a shortened serological procedure for detecting incomplete antibodies to soluble protein antigens has been developed by Coombs and Fiset.[8] In the present paper various sheep cell antisera have been examined, before and after varying degrees of photo-oxidation, for their direct agglutinating action and for their combining capacity, as demonstrated by the indirect antiglobulin sensitization test.

MATERIALS AND METHODS

The anti–red cell sera were produced by intraperitoneal injection of rabbits with a 10 per cent suspension of washed cells. Each injection was composed of 1–1$^1/_2$

ml., and a course consisted of 6–8 injections. The rabbits were bled 8–10 days after the last injection

Anti-Sheep Red Cell Serum.—A pool was made of the second course from two rabbits.

Anti-Fowl Red Cell Serum.—The sera after the first course from four rabbits were pooled—likewise after the second course. A third-course pool was made from a different set of four rabbits. Preinoculation sera served as controls.

All the above sera were heated for $^1/_2$ hour at 56° C. to inactivate complement.

Preparation of Globulin Fraction.—The total globulin fraction was precipitated from unheated serum by adding to the serum an equal volume of saturated ammonium sulfate. The precipitating mixture was left overnight in the cold. The following day the material was centrifuged and the precipitate washed once in a 50 per cent saturated ammonium sulfate solution. The precipitate was recentrifuged and dissolved in saline buffered at pH 7.2 with phosphate buffer. The globulin solution was dialyzed against buffered saline until free of NH_4^+ and then stored at −20° C. until required. The protein concentration was determined by the micro-Kjeldahl method.

Photo-oxidation of Serum or Globulin Solutions.—The method used followed in all main respects that described by Tyler.[1] The serum in the presence of the photosensitizer, eosin Y, was exposed to a powerful source of light in one of the flasks of an enlarged modification of the constant-pressure manometer devised by Dr. M. Dixon and illustrated in Figure 2 of his book.[9] The flasks had a capacity of about 100 ml., and the sidearm pipette a capacity of about 12 ml. Ground-glass taps (including one between the two arms of the U tube) were built in, so that, once the flasks had been mounted, oxygen could be flushed through the apparatus. The apparatus was run in a Warburg bath at 23° C. Light was focused obliquely on the flask from above. Mirrors were placed in the water both underneath and beside the flask to reflect as much of the light as possible onto the flask. The light consisted of a special 12-volt 239-watt bulb set in a powerful reflector. It had previously served as an airplane landing light. The distance between the bulb and the flask containing the serum was 33 inches, and the light intensity at this distance was 300 foot-candles.

Direct Agglutination Test.—The serum or globulin fraction was serially diluted in 0.1-ml. volumes. An equal volume of a 2 per cent suspension of washed sheep cells was then added to each tube, and the contents mixed. Two drops were then removed from each tube and placed in small Rh tubes (rimless glass test tubes, 7 × 50 mm.), in which the cells were allowed to settle at 37° C. After two hours the deposited cells were examined for agglutination in three ways. First, the deposit was observed with the naked eye. Second, some of the sedimented cells were removed with a Pasteur pipette and examined on a glass slide under a microscope using the $^2/_3$-inch objective. Finally, this was repeated after the tube containing the cells had been centrifuged for 10 seconds. Only if this latter procedure demonstrated no agglutination was the serum considered to be without agglutinating action.

Indirect Antiglobulin Sensitization Test.—The main titration tubes of the direct agglutination test (0.2 ml. minus 2 drops) were incubated for 30 minutes at 37° C. Each tube was then centrifuged, and the deposited cells washed twice with saline to remove free rabbit serum. After the second washing, the deposited cells were resuspended in 2 drops of saline. One drop from each tube was then added to

1 drop of a 1:10 dilution of a goat anti-rabbit globulin serum in a Rh tube. The contents of the tubes were mixed, and the test allowed to incubate at 37° C. for $1^1/_2$ hours, while the cells sedimented. The deposited cells were then read for agglutination.

The anti-rabbit globulin serum was from a goat which had received five courses of injections of rabbit serum. The serum after heat inactivation possessed no antibodies for sheep cells and therefore required no absorptions previous to its use in the test.

EXPERIMENTAL RESULTS

Rabbit antisera produced against sheep red cells and fowl red cells were photo-oxidized. Fowl red cells possess the Forssman antigen, which is also present on sheep red cells. Thus rabbit anti-fowl red cell sera may be considered as Forssman antisera. These sera possess a high titer of hemolysin for sheep cells but only a relatively low titer of agglutinin. This divergence in the titer of the hemolysin and agglutinin is due, in the authors' opinion, to the Forssman antigen sites on the sheep cells being situated slightly below the outer periphery of the cell envelope, making it difficult for antibodies to link the cells together. As one of the purposes of this investigation was to produce a nonagglutinating rabbit antibody to sheep cells, it was considered that a Forssman antiserum had much to recommend it as starting material.

The agglutinating and sensitizing capacity of the different sera for sheep cells after varying degrees of photo-oxidation are shown in Table 1. To rule out the possibility that photo-oxidation results in the production of some factor which is

TABLE 1

AGGLUTINATING AND SENSITIZING CAPACITY OF SERA AGAINST SHEEP RED CELLS

Antiserum	Treatment (Mm^2 O_2/Ml)	Titer Producing Agglutination	Titer Producing Sensitization
R.2221-2 (pool); anti-sheep cells; 2d course	Untreated	5,120	20,480
	P.O.* 372	20–1,280	20,480
	657	(20–320)†	20,480
	813	(20–320)	20,480
	1,000	0	10,240
R.2371-4 (pool); anti-fowl red cells; 1st course	Untreated	80	5,120
	P.O. 370	40	640
	420	10	640
	470	(5)	320
	520	0	80
	640	0	5
R.2371-4 (pool); anti-fowl red cells; 2d course	Untreated	160	5,120
	P.O. 350	5 (10)	2,560
	400	0	640
	450	0	160
	500	0	40
R.2281-4 (pool); anti-fowl red cells; 3d course	Untreated	320	5,120
	P.O. 500	10	2,560
	650	0	640
	766	0	320
R.2371-4 (pool); anti-fowl cells; 2d course (Total globulin fraction, $2^1/_2$ per cent)	Untreated	160	10,240
	P.O. 100	5	5,120
	200	0	640
	275	0	320
	325	0	320
	400	0	160

* P.O. = Photo-oxidation.
† Parentheses indicate weak agglutination.

nonspecifically adsorbed onto the red cells and detectable by the antiglobulin technique, preinoculation sera were photo-oxidized and tested in a control series.

With the second-course anti–sheep cell serum, photo-oxidation to the degree of 900–1,000 mm^3 O_2/ml serum was required before all trace of agglutinating capacity was lost. The serum acquired a syrupy consistency as a result of this extensive treatment. When diluted 5,000 times, it was still capable of sensitizing sheep cells. After photo-oxidation to the extent of 657 mm^3 O_2/ml, the serum was no longer capable of showing macroscopic agglutination, but weak agglutination could be shown microscopically over the zone of the $^1/_{20}$–$^1/_{320}$ dilutions of the serum. The prozone is interpretable as due to inhibition by antibody rendered nonagglutinating.

The degree of photo-oxidation required to render the Forssman antisera totally nonagglutinating may be seen in Table 1. The second- and third-course sera were better than the first-course serum in retaining their sensitizing capacity after the agglutinating capacity had been destroyed. The anti-Forssman sera required less photo-oxidation than did the anti–sheep cell serum to be rendered completely nonagglutinating. This may be due to the fact that only a single antigen-antibody system is involved or to the special circumstances covering agglutination by the Forssman antibody. Photo-oxidation of the isolated globulin fraction of the Forssman antiserum also rendered the antibodies nonagglutinating.

It may be seen most clearly with the Forssman antisera that, alongside the conversion of the agglutinating antibody into sensitizing but nonagglutinating antibody, there is also a gradual destruction of the sensitizing capacity of the antibodies. This is most clearly seen with the first- and second-course antisera. For this reason the treatment given to a particular serum must be kept to the minimum compatible with the removal of all agglutinating activity.

DISCUSSION

The results obtained in these experiments show that a nonagglutinating but sensitizing rabbit anti–sheep red cell serum may be obtained with the aid of photo-oxidation. Much more work needs to be done to determine the extent of photo-oxidation required to render different sera nonagglutinating and also to study the effect of hyperimmunization on the resistance of the antibody molecules to the oxidative process.

The uptake of oxygen by the globulin fraction proceeded at a much slower rate than did that of an equivalent amount of the corresponding whole serum, but the extent of photo-oxidation (in cubic millimeters of oxygen per milliliter) required to convert agglutinating into nonagglutinating antibody appears to be roughly proportional to the protein concentration (Table 1). The stability of these photo-oxidized antibodies is another matter which must be studied. Some have been kept over six months in the dark at −20° C. with little if any apparent change in serological reactivity.

The application of these results to the problem of obtaining a sheep red cell protein antigen unit for the test envisaged by Coombs, Howard, and Mynors[6] for the detection of incomplete antibodies to soluble protein antigens has been encouraging. Egg albumin has been successfully coupled by means of tetrazotized benzidine to a photo-oxidized nonagglutinating sheep cell antibody. The photo-

oxidized antibody was then able to carry the coupled egg albumin to the sheep cell surface, giving a stable sheep cell–egg albumin unit capable of detecting either precipitating or nonprecipitating antibodies to egg albumin.[8]

The action of photo-oxidation on antisera has been considered[1] to be one of splitting of the protein molecules and complexing of the different fragments. In view of this, an attempt was made to see whether a protein such as egg albumin could be made to complex with a sheep cell antibody. An approximately equimolecular mixture of egg albumin and protein from the globulin fraction of a Forssman antiserum was photo-oxidized. After an arbitrary degree of oxygen absorption the mixture was found to be capable of sensitizing sheep cells, so that, after washing, they could be powerfully agglutinated by anti–egg albumin serum. This indicated that the sheep cell antibody in the globulin fraction had complexed with the egg albumin. This phenomenon is being investigated more thoroughly in order to determine its possible application to the formation of sheep cell protein antigen units.[6]

SUMMARY

The antiglobulin sensitization test has been found to be effective in detecting the sensitizing capacity of sheep cell antibodies which have been rendered nonagglutinating by the process of photo-oxidation. Such treated antibodies evidently still retain globulin specificity. In the discussion it is mentioned that nonagglutinating rabbit antibodies to sheep cells may be conjugated with a foreign protein such as egg albumin. A sheep cell–egg albumin unit may then be formed by which it is possible to detect serologically complete or incomplete antibodies to egg albumin in sera of man or animals other than the rabbit.

* This investigation was supported in part by a research grant (C-2302) from the National Cancer Institute of the National Institute of Health, Public Health Service.

† This author would like to thank the National Microbiological Institute of the United States Public Health Service for a fellowship during the tenure of which this work was carried out.

[1] A. Tyler, *J. Immunol.*, **51**, 157–172, 329–337, 339–347, 1945.

[2] J. R. Marrack, *The Chemistry of Antigens and Antibodies* ("Medical Research Council Special Report Series," No. 230 [London: H.M. Stationery Office, 1938]).

[3] M. Heidelberger, *Chem. Rev.*, **24**, 323–343, 1939.

[4] L. Pauling, *J. Am. Chem. Soc.*, **62**, 2643–2657, 1940.

[5] R. R. A. Coombs, A. A. Mourant, and R. R. Race, *Brit. J. Expil. Pathol.*, **26**, 255–266, 1945.

[6] R. R. A. Coombs, A. N. Howard, and L. S. Mynors, *Brit. J. Exptl. Pathol.*, **34**, 525–534, 1953.

[7] See *ibid.*, Fig. 1.

[8] R. R. A. Coombs and M. L. Fiset (in press).

[9] M. Dixon, *Manometric Methods* (3d ed.; Cambridge: At the University Press, 1951).

SOME PHYSICAL ASPECTS OF THE STABILITY OF PARALLEL FLOWS*

By C. C. Lin

DEPARTMENT OF MATHEMATICS, MASSACHUSETTS INSTITUTE OF TECHNOLOGY

Communicated by Theodore von Kármán, May 17, 1954

1. *Introduction.*—The problem of hydrodynamic stability has been well known for the difficulty of its mathematical analysis. This is perhaps connected with the inherent intricacy of the physical processes involved, which seem never to have been fully described. Notable among these is the dual role of the viscous forces. On the one hand, viscous forces tend to damp out the oscillations; on the other hand, they are often responsible for the production of a Reynolds shear stress which is actually favorable for the transfer of the energy from the basic flow into the oscillations. The relative magnitude of these two processes determines whether the disturbance will grow or die away. This balance of energy has been treated by Reynolds,[1] Lorentz,[2] Orr,[3] Hamel,[4] von Kármán,[5] Prandtl,[6] and others. Schlichting[7] calculated it explicitly for certain special cases of oscillation in the boundary layer.

Whichever way one may choose to describe the physical process for causing instability, the energy equation must show a rate of increase of the energy of disturbance through the action of the Reynolds stress. Thus a knowledge of its distribution will contribute to our understanding of the mechanism of instability from the energy point of view. The purpose of the present article is an attempt to throw further light on this problem, and it consists of the following studies: (*a*) formulas for the distribution of Reynolds stress in the inviscid case; (*b*) the generation of Reynolds stress at the wall by the viscous forces, including a formula for its magnitude and direction; (*c*) the connection of these results with the more formal mathematical theory.

a) In the inviscid case, the process of stability and instability of parallel flow has been described by the present writer[8] in terms of von Kármán's[9] important mechanism of vorticity redistribution, which was first discussed by him in connection with the vorticity transfer theory of fully developed turbulence. In particular, it was found possible to derive an acceleration formula for vortices by using von Kármán's idea and to use this formula for interpreting Rayleigh's theorem regarding the point of inflection of the velocity profile. The conclusion was that it should be regarded as an extremum for the vorticity distribution. More recently, Rossby[10] used these ideas for the explanation of the motion of the polar air masses, a problem which is fundamental in atmospheric processes.

Despite the satisfaction derived from the vorticity interpretation, it is still desirable to be able to understand the process from the energy point of view. Fortunately, this is possible through the formulas for the distribution of Reynolds stress given by Tollmien[11] (eq. [3.10]) for the neutral case and by Foote and Lin[12] (eqs. [3.3] and [3.6]) for the self-excited case. These formulas will be derived again in section 3, more directly in terms of physical quantities. In particular, Rayleigh's theorem follows directly from these formulas.

b) In the viscous case, known cases of parallel flows, stable according to Rayleigh's criterion but unstable in the presence of viscosity, leave little doubt that viscosity indeed plays an important role in causing instability. Some discussions

of this mechanism are given by Prandtl,[6] in terms of the Reynolds stress produced by the viscous forces through its action in shifting the relative phase of the two velocity components of the oscillation. It is important to know whether this Reynolds stress acts to augment or to retard the disturbance. Unfortunately, the sign of this stress is not given in Prandtl's analysis.

We shall present, in section 2, a more complete analysis of the phase shift (cf. Fig. 1) and shall show that the Reynolds stress (developed through the viscous forces at the wall [eq. (2.10)]) is indeed always favorable for the conversion of energy from the basic flow to the disturbance, if the disturbances are in the form of waves propagating in the *same* direction as the flow velocity near the wall (which is usually the case).

c) Finally, by equating the Reynolds stresses calculated from the above two methods at the edge of the viscous layer near the wall, a relation is obtained (eq. [4.2]) which is prominent in the usual mathematical theory and yet was obtained only after fairly complicated analysis, without any clear indication of its interpretation. To this extent, we have gained some more physical insight into the mathematical analysis. It is hoped that further progress like that presented here will make it possible eventually to present a fairly complete theory in which the physical interpretation of the various steps of the analysis can be more readily followed.

2. *Reynolds Stress Developed by Viscous Forces.*—Let the flow be represented by the components of velocity $(U + u, v)$ in the direction of the co-ordinate axes (x, y), where $U(y)$ is the basic flow and $u(x, y, t)$, $v(x, y, t)$ are the time-dependent components of velocity of the disturbance. The vorticity of the flow is $Z + \zeta$ where $Z = -dU/dy$ and $\zeta = v_x - u_y$. The linearized vorticity equation is

$$\frac{\partial \zeta}{\partial t} + U\frac{\partial \zeta}{\partial x} + v\frac{dZ}{dy} = \nu\left(\frac{\partial^2 \zeta}{\partial x^2} + \frac{\partial^2 \zeta}{\partial y^2}\right), \tag{2.1}$$

where ν is the kinematic viscosity coefficient. We shall consider periodic, wavy disturbances with the exponential factor, $\exp[i(\alpha x - \omega t)]$ and with the amplitudes depending only on y.

It is well known that a periodic oscillation produces a viscous layer near the boundary. In the simplest case of the oscillation of a flat plate parallel to itself, the vorticity distribution is given by the real part of

$$\zeta = Ae^{-i\omega t}\, e^{-(1-i)\eta}, \tag{2.2}$$

where A is a constant, $\eta = y/\delta_0$, and $\delta_0 = (2\nu/\omega)^{1/2}$. The same vorticity distribution obviously holds for a stationary plate and an oscillating stream. In the latter case, the velocity distribution is given by the real part of

$$u = ae^{-i\omega t}\,(1 - e^{-(1-i)\eta}), \qquad \frac{(1-i)a}{\delta_0} = A. \tag{2.3}$$

In the present case, the problem is complicated by the existence of the basic flow $U(y)$ and by the dependence of the disturbance on the co-ordinate x. This latter fact gives rise to a v-component through the equation of continuity:

$$v = -\int_0^y \frac{\partial u}{\partial x}\, dy. \tag{2.4}$$

Thus a Reynolds shear $\tau = -\rho\overline{uv}$ could be developed, where the bar denotes an average with respect to x. We want to determine the sign and magnitude of this Reynolds stress, especially near the wall, $y = 0$.

Let us now consider the oscillations in the flow field, first in the absence of viscosity. There is, in general, a tangential component of the motion at the wall. If the condition for a viscous fluid at the wall is now applied, this represents an infinity of vorticity, which must be spread out by the viscous forces, in accordance with equation (2.1). Now $U = v = 0$ on the wall, and hence equation (2.2), which satisfies equation (2.1) for $U = v = 0$, still describes this diffusion process accurately near the wall. In fact, in the immediate vicinity of the wall,

$$\zeta = Ae^{i(\alpha x - \omega t)}\, e^{-(1-i)\eta} \tag{2.5}$$

would be an adequate periodic solution for equation (2.1) since the term ζ_{xx} is negligible compared with ζ_{yy} within the thin viscous layer.

To calculate the Reynolds stress, we have to obtain the velocity components from the equation

$$v_x - u_y = \zeta \tag{2.6}$$

and the equation of continuity (2.4). We can only obtain the first terms of power series developments near the wall, since (2.5) would not be adequate at finite distances. However, it is exactly the viscous forces near the wall which are responsible for the phase shift causing the shear stress, and we shall find a very clear description of this process by the method of the power series (see Fig. 1).

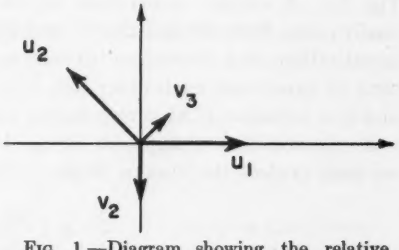


FIG. 1.—Diagram showing the relative magnitude and phase of the various components of velocity.

Let the complex amplitude functions of the disturbance be

$$\left.\begin{aligned} f(y) &= u_1 y + u_2 y^2 + u_3 y^3 + \ldots \\ g(y) &= v_1 y + v_2 y^2 + v_3 y^3 + \ldots \end{aligned}\right\}, \tag{2.7}$$

that is,

$$u = Re\{f(y)e^{i(\alpha x - \omega t)}\}, \qquad v = Re\{g(y)e^{i(\alpha x - \omega t)}\}.$$

The Reynolds stress is given by $\tau = -\rho\overline{uv} = -(p/2)Re(fg^*)$, where the asterisk denotes the complex conjugate. The equation of continuity shows that

$$v_1 = 0, \qquad 2v_2 = -i\alpha u_1, \qquad 3v_3 = -i\alpha u_2, \ldots . \tag{2.8}$$

Then a little calculation gives the Reynolds stress as

$$\tau = -\frac{\rho\alpha}{2}\, y^4 Re(iu_1^* u_2) = -\frac{\rho\alpha}{2}\, |u_1|^2 y^4 Re\left(\frac{iu_2}{u_1}\right),$$

or, by equations (2.7) and (2.8),

$$\frac{\tau}{\rho\overline{v^2}} = -\frac{2}{3\alpha}\, Re\left(\frac{iu_2}{u_1}\right),$$

where $\overline{v^2} = 1/2\ Re(gg^*)$ is the mean-square value of the v-component of the dis-

turbance. The ratio (u_2/u_1) is found by substitution of equation (2.7) into equation (2.6) and by using equation (2.5).[13] One finds easily that

$$\frac{u_2}{u_1} = -\frac{1-i}{2\delta_0}, \qquad Re\left(\frac{iu_2}{u_1}\right) = -\frac{1}{2\delta_0}. \tag{2.9}$$

Thus

$$\frac{\tau}{\rho\overline{v^2}} = \frac{1}{3\alpha\delta_0} > 0. \tag{2.10}$$

We see that the Reynolds stress is indeed *always positive*, i.e., it is a force in the direction of propagation of the wavy disturbance acting on a unit area with positive normal in the direction of the y-axis. The work done on the basic flow $\tau(dU/dy)$ converts energy from the basic flow *into* the disturbance when $dU/dy > 0$.

It is interesting to examine the phase relations of the coefficients u_1, u_2, v_2, and v_3. From equation (2.8) we see that u_1 and v_2, being related by the equation of continuity, are $\pi/2$ out of phase with each other, giving rise to no Reynolds stress (see Fig. 1). A similar observation applies to u_2 and v_3. The Reynolds stress actually arises from the fact that u_1 and u_2 are not in phase (see eq. [2.9]), and consequently there is a correlation between u_1 and v_3 and between u_2 and v_2. Even these tend to cancel out each other (see Fig. 1). The *difference* between the factors 2 and 3 in equation (2.8) is responsible for a net Reynolds stress.

3. *Distribution of Reynolds Stress Away from the Wall.*—Away from the wall, we may neglect the viscous terms. Then the vorticity equation (2.1) becomes

$$\frac{\partial\zeta}{\partial t} + U\frac{\partial\zeta}{\partial x} + v\frac{dZ}{dy} = 0. \tag{3.1}$$

If the vorticity is in the form of an amplified wavy disturbance

$$\zeta = Ae^{\lambda t}\sin(\alpha x - \omega t + \epsilon), \tag{3.2}$$

where A and ϵ are functions of y, then equation (3.1) gives

$$v = -\left(\frac{dZ}{dy}\right)^{-1} Ae^{\lambda t}\left\{\lambda\sin(\alpha x - \omega t + \epsilon) + (\alpha U - \omega)\cos(\alpha x - \omega t + \epsilon)\right\}. \tag{3.3}$$

The Reynolds stress τ is well known to be related to the vorticity transfer by the relation

$$\frac{1}{\rho}\frac{d\tau}{dy} = \overline{v\zeta}. \tag{3.4}$$

By equations (3.2) and (3.3), this becomes

$$\frac{1}{\rho}\frac{d\tau}{dy} = -\frac{\lambda}{2}\left(\frac{dZ}{dy}\right)^{-1} A^2e^{2\lambda t},$$

or

$$\frac{1}{\rho\overline{v^2}}\frac{d\tau}{dy} = -\frac{dZ}{dy}\frac{\lambda}{(\alpha U - \omega)^2 + \lambda^2}. \tag{3.5}$$

The terms in the right-hand side of this equation are known. In terms of quantities familiar in the usual stability theory, equation (3.5) may be written as

$$\frac{1}{\rho\overline{v^2}}\frac{d\tau}{dy} = \frac{c_i U''/\alpha}{(U - c_r)^2 + c_i^2}, \qquad \text{where} \qquad \omega = \alpha c_r, \lambda = \alpha c_i. \tag{3.6}$$

In the limit $\lambda \to 0$ these equations give τ = constant, except at $U = c_r$. Thus, for neutral oscillations, the Reynolds stress remains constant except for a possible jump at the critical layer, where the flow speed is equal to the wave speed. The extent of this jump can be calculated from equation (3.5) or equation (3.6) as follows: We regard U as the independent variable in equation (3.6) and obtain

$$\tau_2 - \tau_1 = \int_{U_1}^{U_2} F(U) \frac{c_i}{(U - c_r)^2 + c_i^2} dU, \tag{3.7}$$

where

$$F(U) = -\frac{\rho\overline{v^2}}{\alpha}\frac{dZ}{dU}. \tag{3.8}$$

In the limit $c_i \to 0$, a familiar theorem on potential theory[14] shows that

$$\tau_2 - \tau_1 = \pi F(c_r), \tag{3.9}$$

if $U_2 > c_r > U_1$. Thus

$$[\tau] = \tau(c + 0) - \tau(c - 0) = -\frac{\pi}{\alpha}\rho\left(\overline{v^2}\frac{dZ/dy}{dU/dy}\right)_c, \tag{3.10}$$

where the subscript c denotes the critical point $U = c$ and $[\tau]$ denotes the change of τ from $U = c - 0$ to $U = c + 0$. In dimensionless form, the discontinuity is given by

$$\left[\frac{\tau}{\rho\overline{v^2}}\right] = -\pi\left(\frac{dZ}{dU}\right)_c = -\frac{\pi}{\alpha}\left(\frac{dZ/dy}{dU/dy}\right)_c. \tag{3.11}$$

Relation (3.10) was first obtained by Tollmien,[11] using the concept of the Wronskian determinant. The more general relation (3.6) was obtained by Foote and Lin.[12] It is believed that the present discussion is more tractable from the physical standpoint.

The application of the above results to the derivation of Rayleigh's theorem is very simple. At the wall, or the center line of a channel, or at large distances from a boundary layer, the Reynolds stress must vanish. The application of the boundary conditions at both walls (say) shows that the total *change* of τ must vanish. For self-excited oscillations it can be seen from equation (3.5) that this cannot be true unless the vorticity gradient dZ/dy changes sign; i.e., the velocity distribution must have an inflection point. In the neutral case, equation (3.10) shows that $(dZ/dy)_c = 0$ if $[\tau] = 0$. Thus the neutral oscillation must have a wave speed equal to that of the basic flow at the point of inflection—a relation obtained by Lord Rayleigh in a more mathematical manner.

4. *The Distribution of Reynolds Stress across the Whole Region.*—By combining the above discussions, we may form an over-all picture of the Reynolds stress and

its influence on the transfer of energy. Let us first consider a neutral oscillation in the case of the boundary layer. At the outer edge the Reynolds shear must vanish. As we proceed inward, the shear remains zero, until the critical point is reached. The shear then becomes *positive*, by equation (3.11) (Fig. 2). This picture, based on the inviscid analysis of section 3, will be modified somewhat by the effect of viscosity, but the qualitative behavior should be the same. As the wall is approached, τ remains finite, according to the inviscid analysis, but it must drop to zero at the wall. We see that this would have been impossible if it were not for the mechanism discussed in section 2. A rapid rise of the Reynolds shear from zero to a *positive* value is provided by the phase shift due to viscous forces.

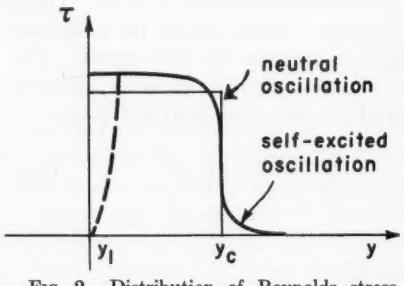


FIG. 2.—Distribution of Reynolds stress (dashed line gives influence viscous forces).

Clearly the distribution of stress described above leads to a net transfer of energy *into* the disturbance. This energy is dissipated into heat by the viscous forces.

The distribution of shear stress in a self-excited disturbance is also shown in Figure 2. Because $d\tau/dy$ is negative for the major part of the flow, τ rises to higher values near the wall than in the previous case. The additional transfer of energy increases the amplitude of the oscillation.

If we now attempt to equate (up to a factor of the order of unity) the stresses obtained from both the above considerations, at the edge of the inner viscous layer (defined by $y = y_1 = k\delta_0$, $k \sim 1$), we shall be led to a relation prominent in the mathematical theory of hydrodynamic stability. Indeed, for the neutral case, we have, by equations (3.10) and (2.10)

$$\left.\begin{aligned} \frac{\tau}{\rho\overline{v^2}} &= -\frac{\pi}{\alpha}\frac{U_c''}{U_c'} \\ \frac{\tau}{\rho\overline{v_1^2}} &= \frac{1}{3\alpha\delta_0} \end{aligned}\right\}, \tag{4.1}$$

where v_1 is the v-component of the disturbance at the edge $y = y_1$ of the inner viscous layer. Since the region between y_1 and y_c is essentially outside the viscous region but still close to the boundary, we may expect the oscillation amplitude to vary linearly, and we obtain

$$\frac{\overline{v_c^2}}{\overline{v_1^2}} = \left(\frac{y_c}{y_1}\right)^2 = \left(\frac{c/U_c'}{k\delta_0}\right)^2.$$

Thus equation (4.1) gives

$$-\frac{\pi c U_c''}{U_c'} = k^2\frac{U_c'\delta_0}{3c}. \tag{4.2}$$

This relation, with $k = \sqrt{3}$, may be easily identified with the imaginary part of the secular equation in the usual mathematical theory (see Lin,[8] eq. [2.9]). It is inter-

esting that such a relation, first obtained by lengthy mathematical analysis, can be approximately reproduced by relatively crude physical arguments.

The left-hand side of equation (4.2) depends on c and the form of the profile alone. It indeed gives a measure of the jump of Reynolds stress in the following form, as can be easily verified by using equation (4.1)

$$-\frac{\pi c U_c''}{U_c'^2} = \frac{\omega}{U_c'}\left[\frac{\tau}{\rho\overline{v^2}}\right]. \tag{4.3}$$

This quantity has been made the basis for the derivation of an estimation formula for the minimum critical Reynolds number, which has been used with considerable success. Again, here it appears through the calculations of Reynolds stress and other general physical considerations.

* This work is sponsored by the Mechanics Branch of the Office of Naval Research.

[1] O. Reynolds, *Phil. Trans.*, *A*, **186**, 123–164, 1895; or *Sci. Papers*, **2**, 535.
[2] H. A. Lorentz, *Abhandlungen über theoretische Physik*, Vol. 1 (Leipzig, 1907).
[3] W. M. F. Orr, *Proc. Roy. Irish Acad. A*, **17**, 124, 1907.
[4] G. Hamel, *Göttinger Nachr.*, pp. 261–270, 1911.
[5] Th. v. Kármán, *Proc. 1st Intern. Congr. Appl. Mech.* (Delft, 1924), p. 97.
[6] L. Prandtl, article in Durand, *Aerodynamic Theory*, Vol. **3**, particularly pp. 186–187.
[7] H. Schlichting, *Nachr. Ges. Wiss. Göttingen*, **1**, 47–78, 1935.
[8] C. C. Lin, *Quart. Appl. Math.*, **3**, 117–142, 218–234, 277–301, 1945.
[9] Th. v. Kármán, *Proc. 4th Intern. Congr. Appl. Mech.* (Cambridge, 1934).
[10] C.-G. Rossby, *J. Meteorol.*, **6**, 163–180, 1949.
[11] W. Tollmien, Nachr. Ges. Wiss. Göttingen, (N.F.), **1**, 79–114, 1935.
[12] J. R. Foote and C. C. Lin, *Quart. Appl. Math.*, **8**, 265–280, 1950.
[13] There should be a small modification due to the term $U\zeta_x$ in eq. (2.1).
[14] $\lim_{y \to 0} \int_{\xi_1}^{\xi_2} \frac{y}{(x-\xi)^2 + y^2} f(\xi)\, d\xi = \pi \int_{\xi_1}^{\xi_2} \delta(x-\xi) f(\xi)\, d\xi, \ (y > 0).$

SYMPOSIUM ON THE STRUCTURE AND FUNCTION OF NUCLEIC ACIDS

BY LINUS PAULING

GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated June 25, 1954

During recent years there have been several significant advances in the understanding of the chemical nature, molecular structure, and biological function of the nucleic acids. At the request of President Detlev Bronk, Sterling B. Hendricks arranged a symposium on this subject for the afternoon of April 26, 1954, the first day of the annual meeting of the Academy.

The symposium was opened by a brief introduction by the chairman, Linus Pauling. Professor Alexander R. Todd of Cambridge University then discussed the chemistry of the nucleic acids, including the important discoveries made by him and his students. The Watson-Crick model of the deoxyribonucleic acid mole-

cule and its biological implications were discussed in talks by F. H. C. Crick and J. D. Watson, and studies on the biosynthesis of nucleic acid were presented by Ellis Bolton. These four papers are reproduced in the following pages. The symposium was concluded by two additional informative papers: "Genetic Functions of Desoxyribonucleic Acids in Bacterial Transformations," by Rollin D. Hotchkiss, Rockefeller Institute for Medical Research, and "Nucleic Acid and Viral Growth," by A. D. Hershey, Carnegie Institution of Washington, Cold Spring Harbor.

CHEMICAL STRUCTURE OF THE NUCLEIC ACIDS

BY ALEXANDER R. TODD

CHEMICAL LABORATORY, UNIVERSITY OF CAMBRIDGE

Communicated by Linus Pauling, May 7, 1954

During the last four years a remarkable transformation has occurred in our knowledge of the nucleic acid structure, and there is reason to believe that many of the major features may now be regarded as established. It is the purpose of this brief review to outline the main developments which have taken place and to indicate the chemical evidence upon which they rest.

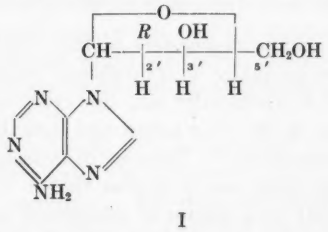
The known nucleic acids are of two types—the ribonucleic acids, yielding upon total hydrolysis the sugar D-ribose, and the deoxyribonucleic acids, yielding 2-deoxy-D-ribose. Graded hydrolysis of nucleic acids gives first the nucleotides and then the nucleosides. From ribonucleic acids, four nucleosides—adenosine, guanosine, uridine, and cytidine—are obtained, and from deoxyribonucleic acids, six—deoxyadenosine, deoxyguanosine, deoxycytidine, thymidine, 5-methyl-deoxycytidine, and 5-hydroxymethyl-deoxycytidine—of which the two last-named appear to be of limited occurrence. Schematically, the hydrolytic breakdown of the nucleic acids may be represented thus:

Nucleic acids
↓
Nucleotides
↓
Nucleosides + Phosphoric acid
↓
Purines or pyrimidines + D-ribose or 2-deoxy-D-ribose

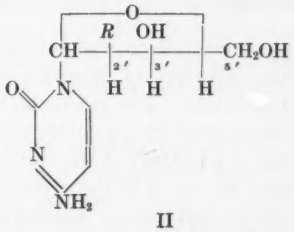
Ever since it was realized that the nucleotides are phosphates of the nucleosides and that the nucleic acids can be regarded as polynucleotides, many attempts have been made to formulate general structures for the latter. Titrimetric studies by Levene and Simms[1] led to a general concept of nucleic acid structure in which the individual nucleoside residues were joined together by phosphodiester linkages, and eliminated from consideration ether or pyrophosphate linkages. This simple formulation has become generally accepted, and, although other types of linkage have been suggested from time to time, such suggestions lack corroborative chemical evidence. Of these suggestions, the phosphotriester linkage, which might represent a branching point in a polynucleotide, has perhaps been most widely canvassed, and

it will be mentioned later in connection with the branching problem. Essentially, however, the nucleic acids may be regarded as polydiesters of phosphoric acid.

It is clear that an understanding of the details of nucleic acid structure must rest upon a knowledge of the precise structure of the individual nucleosides, and of the position, as well as the nature, of the internucleotidic linkage. These problems my colleagues and I in Cambridge have endeavored to study, using the methods of organic chemistry, and I believe that a consideration of the development of our studies and of related biochemical work affords the simplest exposition of nucleic acid structure as we know it today. The earlier work of Levene, in particular, established some of the general features of the group and simplified our studies at least in their earlier phases. As a preliminary phase in our work, we extended earlier studies by establishing in every detail the structure and stereochemical configuration of the natural ribonucleosides and confirmed our findings by total synthesis. They are in each case β-D-ribofuranosides, the sugar residue being attached at N_9 in the purine nucleosides and at N_3 in the pyrimidine nucleosides;[2] typical examples are adenosine (I in the accompanying diagram; R = OH), and cytidine (II; R = OH). The total synthesis of the deoxyribonucleosides has not yet been achieved, but their structure as β-2-deoxy-D-ribofuranosides with the sugar attached at N_9 in the purine and N_3 in the pyrimidine nucleosides has been rigidly established in the cases of deoxyadenosine (I; R = H), deoxycytidine (II; R = H), and thymidine.[3] In the case of deoxyguanosine the β-configuration, although allotted by analogy with the others, is almost certainly correct.



I

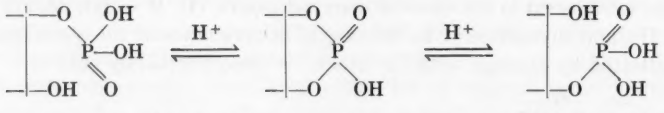


II

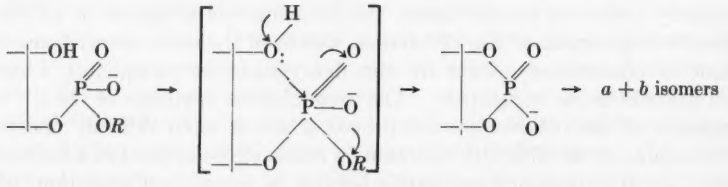
The next step in the direction of the determination of nucleic acid structure was clearly the synthesis of the various simple nucleotides derivable from the nucleosides by the introduction of phosphate groups into the glycosidic portion of the molecule. For this purpose a number of new and flexible methods of phosphorylation were devised,[2] the most successful involving the use of dibenzyl phosphorochloridate $[(C_6H_5CH_2O)_2POCl]$[4] as phosphorylating agent. Using it with appropriately protected intermediates, the unambiguous synthesis of all the ribonucleoside-5′-phosphates[5] was effected, as well as of the main deoxyribonucleoside-3′- and -5′-phosphates[6] (except for the deoxyguanosine phosphates, whose synthesis has still to be completed). The unambiguous synthesis of the 2′- and 3′-phosphates of the ribonucleosides proved a much more difficult undertaking, partly because of the difficulty of obtaining ribonucleosides blocked simultaneously in the 3′- and 5′-positions and partly because of phosphoryl migration, which is discussed below. Brown and Todd[7] were able to prepare adenosine-2′-phosphate and adenosine-3′-phosphate by phosphorylating 5′-trityladenosine, but they were unable at the time to say which was the 2′- and which the 3′-compound; the same

was, of course, true of other ribonucleoside 2′- and 3′-phosphates prepared in the same way from the appropriate 5′-trityl derivatives.

Until 1949 it had been believed that alkaline hydrolysis of ribonucleic acids yielded only four nucleotides, which were regarded on evidence, now known to be of doubtful validity, as the 3′-phosphates of the four nucleosides. In that year, however, Carter and Cohn[8] applied ion-exchange chromatography to alkaline hydrolyzates of ribonucleic acids and showed that they contained not four but eight nucleotides made up of four pairs of isomers—an *a* and a *b* nucleotide corresponding to each of the four ribonucleosides. Since it is clear, on the basis of evidence of many sorts, that the four *a* nucleotides all have the phosphate residue in the same position and the *b* nucleotides likewise, it simplifies matters to discuss the problem in one specific case—the adenylic acids *a* and *b*—bearing in mind that what is said here about this pair applies equally to each of the other pairs. The synthetic 2′- and 3′-phosphates of adenosine were shown to be identical with adenylic acids *a* and *b*, although, as already mentioned, it could not then be decided which was 2′- and which was 3′-phosphate. Brown and Todd[7] observed that, although quite stable in alkali, acid solutions of either isomer undergo phosphoryl migration, giving an equilibrium mixture of both. This interconversion of the *a* and *b* nucleosides can be written formally as follows:



Even more interesting was the behavior of simple monoesters of the *a* and *b* nucleotides. Diesters of phosphoric acid are normally very resistant toward alkaline hydrolysis. The esters of the *a* and *b* nucleotides are, however, very labile to alkali, undergoing hydrolysis with loss of the ester group and formation in each case of a mixture of the three *a* and *b* nucleotides.[7] This ease of hydrolysis is also shown by esters of the glycerophosphoric acids, and it is clear that in such cases the structural feature causing alkali-lability in a diester of phosphoric acid is the presence of a *cis*-hydroxyl group on the carbon atom adjacent to the point of attachment of the phosphate residue in one of the esterifying groups. The process of hydrolysis postulated is indicated schematically below. The formation of the cyclic phosphate as an intermediate has been demonstrated, and such cyclic phosphates have been synthesized[9] in the nucleotide series and shown to have the expected properties.



It should be noted here that the expulsion of the esterifying group *R* is an inevitable consequence of the cyclization step—one of the esterifying groups clearly must be removed if the phosphorus atom is to retain its normal valency, and mere

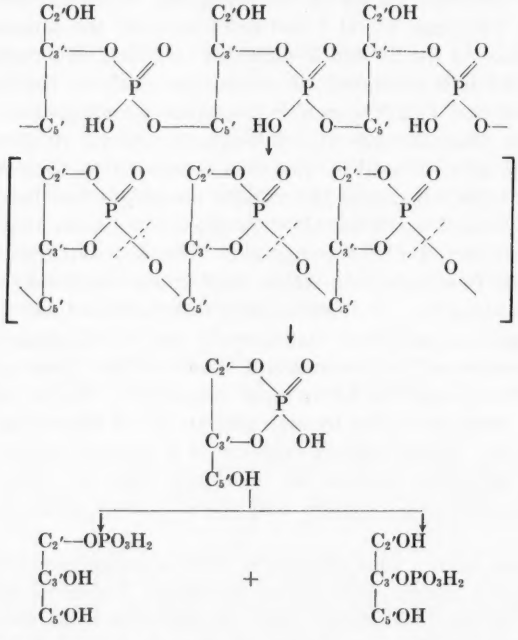
rupture of either linkage with the nucleoside residue would not give any degradation of the molecule; a neutral cyclic triester of phosphoric acid is not an intermediate in the process (although it may at times be convenient to write the intermediate as such in formal schematic representations of the process in complex molecules). The production of a mixture of *a* and *b* nucleotides upon hydrolysis of the cyclic phosphate is to be expected, since the two ester linkages in the latter are effectively equivalent.

Although the structural theory of the ribonucleic acids was developed during the period when the terms *a* and *b* had to be used for the isomeric nucleotides, pending a resolution of the 2′- and 3′-phosphate problem, this resolution has now been achieved, and it is convenient to record the result at this point. Brown, Fasman, Magrath, and Todd[10] were able to prepare a homogeneous *x*:5′-diacetyladenosine and to phosphorylate it. Subsequent removal of protecting groups yielded *exclusively* adenylic acid *a;* this demonstrated that their efforts to avoid any phosphoryl migration during the various operations had been successful (if not, a mixture of *a* and *b* would have been produced) and hence that their diacetyladenosine was, in fact, the *b*:5′-compound. The diacetyl derivative was now tosylated, and the position of the stable tosyl group was rigidly established by degradative methods as $C_{2'}$. It follows rigidly that the diacetyladenosine used was 3′:5′-diacetyladenosine and hence that adenylic acid *a* is adenosine-2′-phosphate. It was also demonstrated by Cochran and Woolfson[10] by X-ray crystallographic analysis that adenylic acid *b* is adenosine-3′-phosphate. Khym and Cohn[11] have also reached the same conclusion by an ingenious use of differential hydrolysis on ion-exchange resins. Much indirect evidence of a physical nature has also been advanced which generally confirms the conclusion that the other *a* nucleotides are also ribonucleoside-2′-phosphates and the *b* isomers ribonucleoside-3′-phosphates.[12]

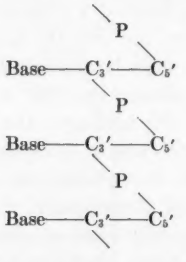
It has long been known that ribonucleic acids undergo very ready hydrolysis with alkali, yielding simple nucleotides; no larger fragments have ever been characterized. The deoxyribonucleic acids, on the other hand, are comparatively stable to alkali and are not degraded to simple nucleotides. As a result of their observations on the alkali-lability of the esters of *a* and *b* nucleotides, Brown and Todd[13] were able to advance a simple explanation of the hydrolytic behavior of the nucleic acids and thence to develop a general theory of nucleic acid structure. Structurally the polynucleotides are strictly analogous to the nucleotide esters (the esterifying group in their case is a polynucleotide chain) and must behave similarly on hydrolysis. The scheme (p. 752) represents the alkaline hydrolysis of a ribonucleic acid based on this analogy; the scheme predicts total hydrolysis exclusively to a mixture of the 2′- and 3′-phosphates of the respective nucleosides, which is, in fact, observed. In the scheme, the expression $C_{2'}$—$C_{3'}$—$C_{5'}$ is used to represent the sugar residue of a nucleoside, the hydroxyls in the 2-, 3-, and 5-positions of the chain being the only ones concerned in linkages or reactions.

The reason for the alkali-stability of the deoxyribonucleic acids is at once apparent—having no hydroxyl group at C_2, they cannot undergo the cyclization process, and so they show the normal stability of simple diesters of phosphoric acid. It is interesting to reflect that easy alkaline hydrolysis depends on the sugar in a nucleic acid being ribose—no other pentose would satisfy the structural re-

quirement for lability. This mechanism of hydrolysis does not of itself fix the internucleotidic linkage in ribonucleic acids, although it requires that one point of attachment be C_2 or C_3. The 3′:5′ linkage shown in the scheme was preferred to the 2′:5′ in the original postulate[13] mainly by analogy with the deoxyribonucleic acids, in which only the 3′:5′ linkage is possible, and it has been fully justified by later work. It is convenient at this point to discuss the structural features of the two types of acid separately.



Deoxyribonucleic Acids.—These acids are formulated as linear polynucleotides with a recurring 3′:5′ phosphodiester linkage, as shown below:[13]

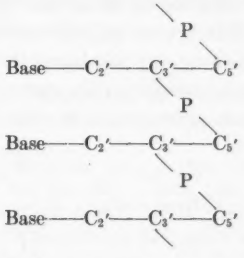


This formulation accords with their stability toward alkali and with the fact that the nucleotides formed on enzymic hydrolysis have been shown to be nucleoside-5′-phosphates by enzymic methods[14] and by synthesis.[15] The production of 3′:5′-diphosphates of the pyrimidine nucleosides on acid hydrolysis[16] is also in

accord with this structure and shows the participation of both $C_{3'}$ and $C_{5'}$ in the internucleotidic linkage. The initial action of acid on deoxyribonucleic acids is to hydrolyze the purine glycosidic linkages, yielding the apurinic acids.[17] In the second phase of its action these apurinic acids are degraded mainly by elimination reactions rather than by hydrolysis, since each purine-free sugar residue in them has a phosphate linkage at $C_{3'}$, i.e., in the β-position to a carbonyl or potential carbonyl group. In this way pyrimidine nucleoside-3′:5′-diphosphates would be expected to arise from those sites in the original molecule where purine and pyrimidine nucleoside residues were adjacent.

The alkali-stability of the deoxyribonucleic acids would seem to preclude branched-chain structures. The only type of branching which could be expected would be on phosphorus, and the resulting phosphotriester linkages would be unstable to alkali. So far no method has been devised to determine the sequence of residues in deoxyribonucleic acids.

Ribonucleic Acids.—Brown and Todd[13] postulated a recurring 3′:5′-linked polynucleotide structure for the main chain in ribonucleic acids, i.e.,

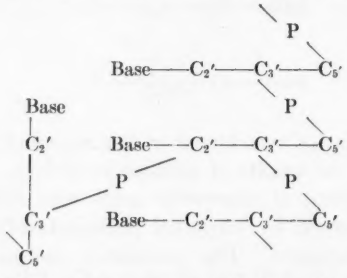


That $C_{2'}$ or $C_{3'}$ is one point of attachment of the internucleotidic linkage follows, as indicated above from the results of alkaline hydrolysis. $C_{5'}$ is also involved, since, for example, treatment of ribonucleic acids with snake-venom diesterase[18] or with ribonuclease followed by intestinal phosphatase[19] yields large amounts of the nucleoside-5′-phosphates. The postulated mechanism of alkaline hydrolysis has been further confirmed by the actual isolation of cyclic nucleoside-2′:3′-phosphates in very mild barium carbonate and ammonia hydrolyzates.[20] Convincing evidence in support of this structure has come from enzyme studies, particularly from those using ribonuclease.

Crystalline pancreatic ribonuclease acts on ribonucleic acids to yield a variety of products. Short action yields, in addition to larger fragments, substances which have been identified as the cyclic 2′:3′-phosphates of cytidine and uridine.[21] Further action yields the pyrimidine nucleoside-3′-phosphates and a variety of small polynucleotides, mainly di- and tri-, in which the terminal residue bearing the phosphorus at $C_{3'}$ (or in a 2′:3′-cyclic group) is always a pyrimidine nucleoside residue.[22] This apparent specificity for linkages attached to pyrimidine nucleosides has been confirmed and defined by studies of the enzyme's action on synthetic nucleotide esters[23] and on nucleoside 2′:3′-cyclic phosphates.[24] It was found that ribonuclease attacks the cyclic 2′:3′-phosphates of uridine and cytidine, yielding exclusively the 3′-phosphates; it has no action on the corresponding adenosine and guanosine derivatives. Moreover, although it has no action on

simple esters of pyrimidine or purine nucleoside-5′-phosphates or on esters of purine nucleoside-2′- or -3′-phosphates or of pyrimidine nucleoside-2′-phosphates, it hydrolyzes esters of pyrimidine nucleoside-3′-phosphates smoothly to the nucleoside-3′-phosphates, the cyclic 2′:3′-phosphates being detectable as intermediates in the process. From these results it follows that the pyrimidine nucleoside residues in ribonucleic acid have the phosphate group of the internucleotidic linkage at $C_{3'}$ and that ribonuclease acts in a similar way to alkali, except that it attacks only pyrimidine nucleotide sites in the nucleic acid and yields $C_{3'}$-nucleotides rather than a mixture of $C_{2'}$ and $C_{3'}$. Similar studies of the action of spleen nuclease (which attacks both purine and pyrimidine nucleotide sites in polyribonucleotides) on simple nucleotide esters show that the purine nucleoside residues are also linked at $C_{3'}$.[25] It would therefore seem that the postulated 3′:5′-linked polynucleotide structure for the main chain of the ribonucleic acids can be taken as established, and all other evidence obtained in recent years points in the same direction. There is no evidence for any other type of internucleotidic linkage.

The possibility that some ribonucleic acids have a branched-chain structure has been frequently canvassed. Bearing in mind the primary requirement of alkali-lability in any ribonucleic acid structure and the mechanism of alkaline hydrolysis, it is clear that, apart from branching on phosphorus through phosphotriester linkages which would be theoretically admissible, only one type of branching from $C_{2'}$ in nucleoside residues in the main polynucleotide chain is possible,[13] viz.,



In this type of branching, $C_{2'}$ in one residue in the main chain is joined by a phosphodiester linkage to $C_{3'}$ in the first residue of the branching chain. Linkage to $C_{5'}$ in the first residue of the branch would give a structure in which neither that residue nor the branching residue in the main chain would contain a free hydroxyl so situated as to permit the cyclization step in alkaline hydrolysis; as a result, alkaline hydrolysis of a polynucleotide containing such a structure would yield fragments larger than simple nucleotides. There has been much discussion as to whether ribonucleic acids are branched or unbranched, and no decisive evidence either way has been adduced. It may be that both branched and unbranched structures occur, but at the moment one can only say that chemical considerations, although they indicate *how* branching may occur, are silent on whether it does occur. Physical studies or the further progress of synthetic work may decide this important point.

There remains, of course, the problem of the sequence of nucleoside residues in the ribonucleic acids, for it is in the sequence of residues that the difference between the various acids doubtless lies. A method suitable for this purpose has been proposed by Brown, Fried, and Todd.[26] This method depends on the oxidation of a terminal nucleoside residue containing a free 2′:3′-α-glycol grouping with

periodate, followed by expulsion of the oxidized residue under very weakly alkaline reaction by means of an elimination reaction, leaving the rest of the molecule intact. Its validity has been tested in a number of oligonucleotides,[27] but it is clear that it cannot be applied to the nucleic acids themselves until a truly homogeneous ribonucleic acid can be made available. It is worthy of mention that, given a homogeneous ribonucleic acid, this stepwise degradation method of sequence determination should in theory serve to establish the presence or absence of branching; on degrading the main chain, the method would come to a halt when a branching point was reached, since there would be no free α-glycol system in the residue exposed at that point, and hence periodate action would not occur.

This review of present chemical evidence on nucleic acid structure is necessarily incomplete, for it is impossible in a limited space to include all the mass of work which has been done in the field. Nevertheless, I have endeavored to select the main features, in an attempt to give a rational account of our present views and the basic evidence for them. Certain aspects which require further investigation have been indicated, but it seems reasonable to conclude that the broad outline of the chemical structure of this important group of natural substances is now clear enough to open the way to new advances in our knowledge of their behavior and biological functions.

[1] P. A. Levene and H. S. Simms, *J. Biol. Chem.*, **65**, 519, 1925; **70**, 327, 1927.

[2] For a collected account of this work see, e.g., G. W. Kenner, *Fortschr. Chem. org. Naturstoffe*, **8**, 96, 1951.

[3] D. M. Brown and B. Lythgoe, *J. Chem. Soc.*, p. 1990, 1950; W. Andersen, D. H. Hayes, A. M. Michelson, and A. R. Todd, *J. Chem. Soc.*, p. 1882, 1954.

[4] F. R. Atherton, H. T. Openshaw, and A. R. Todd, *J. Chem. Soc.*, p. 382, 1945.

[5] J. Baddiley and A. R. Todd, *J. Chem. Soc.*, p. 648, 1947; A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, p. 2476, 1949.

[6] A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, p. 951, 1953; p. 34, 1954; D. H. Hayes, A. M. Michelson, and A. R. Todd, *J. Chem. Soc.*, 1954 (in press).

[7] D. M. Brown and A. R. Todd, *J. Chem. Soc.*, p. 44, 1952.

[8] C. E. Carter and W. E. Cohn, *Federation Proc.*, **8**, 190, 1949.

[9] D. M. Brown, D. I. Magrath, and A. R. Todd, *J. Chem. Soc.*, p. 2708, 1952.

[10] D. M. Brown, G. D. Fasman, D. I. Magrath, A. R. Todd, W. Cochran, and M. M. Woolfson, *Nature*, **172**, 1184, 1953; *J. Chem. Soc.*, p. 1448, 1954.

[11] J. X. Khym and W. E. Cohn, *J. Am. Chem. Soc.*, **76**, 1818, 1954.

[12] *Cf.*, e.g., L. F. Cavalieri, *J. Am. Chem. Soc.*, **74**, 5804, 1952; J. J. Fox, L. F. Cavalieri, and N. Chang, *J. Am. Chem. Soc.*, **75**, 4315, 1953; A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, p. 34, 1954; H. S. Loring, M. L. Hammell, L. W. Levy, and H. W. Bortner, *J. Biol. Chem.*, **196**, 821, 1952.

[13] D. M. Brown and A. R. Todd, *J. Chem. Soc.*, p. 52, 1952.

[14] C. E. Carter, *J. Am. Chem. Soc.*, **73**, 1537, 1951.

[15] A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, p. 951, 1953; p. 34, 1954; and as yet unpublished data.

[16] C. A. Dekker, A. M. Michelson, and A. R. Todd, *J. Chem. Soc.*, p. 947, 1953.

[17] C. Tamm, M. E. Hodes, and E. Chargaff, *J. Biol. Chem.*, **195**, 49, 1952.

[18] W. E. Cohn and E. Volkin, *Arch. Biochem. and Biophys.*, **35**, 465, 1952.

[19] W. E. Cohn and E. Volkin, *Nature*, **167**, 483, 1951.

[20] R. Markham and J. D. Smith, *Biochem. J.*, **52**, 552, 1952.

[21] R. Markham and J. D. Smith, *Research (London)*, **4**, 344, 1951; *Nature*, **168**, 406, 1951.

[22] R. Markham and J. D. Smith, *Biochem. J.*, **52**, 558, 1952.

[23] D. M. Brown and A. R. Todd, *J. Chem. Soc.*, p. 2040, 1953.

[24] D. M. Brown, C. A. Dekker, and A. R. Todd, *J. Chem. Soc.*, p. 2715, 1952.

[25] D. M. Brown, L. A. Heppel, and R. J. Hilmoe, *J. Chem. Soc.*, p. 40, 1954.

[26] D. M. Brown, M. Fried, and A. R. Todd, *Chemistry & Industry*, p. 352, 1953.

[27] P. R. Whitfeld and R. Markham, *Nature*, **171**, 346, 1953.

THE COMPLEMENTARY STRUCTURE OF DNA

By F. H. C. Crick

PROTEIN STRUCTURE PROJECT, POLYTECHNIC INSTITUTE OF BROOKLYN*

Communicated by Linus Pauling, May 7, 1954

This paper reviews very briefly the recent work on the "structure" of the sodium salt of desoxyribonucleic acid (DNA). By structure is meant not the chemical formula but the spacial arrangement of the atoms. The structure revealed by X-rays is the general structure—that is, the features common to DNA from different sources, since the X-rays only respond clearly to the repeating part of the structure and effectively say nothing about the exact sequence of the bases, which probably does not repeat.

The general chemical formula has been described in these Proceedings by Professor A. R. Todd[1] and will be taken as known. The X-ray work is carried out on fibers drawn from extracted DNA from a variety of sources. The early studies were by Astbury and Bell.[2] Almost all the recent work has been done at King's College, London, by Drs. Wilkins and Franklin and their colleagues. Preliminary studies are reported in Wilkins, Gosling, and Seeds.[3] More detailed reports on the X-ray data have been given in two papers by Franklin and Gosling.[4] Evidence for the helical nature of the structure and for some of its other features has been presented in a preliminary way by Wilkins, Stokes, and Wilson[5] and by Franklin and Gosling.[6] More detailed interpretations of the crystalline form have been given by Franklin and Gosling[7] and by Wilkins, Seeds, Stokes, and Wilson.[8] In addition, Wilkins and Randall[9] have shown that the oriented sperm heads give a very similar X-ray pattern to that from extracted DNA.

The interpretation of their results offered by the experimentalists is in broad agreement with the type of structure proposed by Watson and Crick,[10] though the precise dimensions of this model may be incorrect. A more detailed description of the proposed structure and an account of the methods used in arriving at it have been given by Crick and Watson.[11] As is well known, this structure consists of two helical phosphate-sugar chains, winding round the same axis, the chains running in opposite directions. The chains are linked together by hydrogen bonds between their bases, a base from one chain being paired off with the opposite base on the other chain.

It is postulated that specific pairing occurs and that the only pairs that will normally fit into the structure are

adenine with thymine,
guanine with cytosine (or its derivatives).

The model places no restriction on the sequence of bases along a single chain. If the sequence of the bases on one chain were known, the sequence on the other chain could be written down because of the specified pairing. Thus each chain, with its bases, can be regarded as the "complement" of the other.

The salient features of this structure are as follows:

a) There are two chains in the structural unit.[12] This is strongly suggested by the observed density (which rules out three chains). Model-building shows that a single-chain structure is unlikely. The X-ray data also show "two-ishness."

b) The chains are arranged helically. This was originally postulated as the obvious way to explain the large dimensions of the unit cell. It has now received overwhelming support from the detailed X-ray data, interpreted in the light of the helical diffraction theory of Cochran, Crick, and Vand[13] (also Stokes, unpublished).

c) The chains are held together by specific pairs of bases. It is not generally realized that this feature was not assumed originally in the model-building but was introduced as the only way of joining the two chains together which was structurally plausible. Moreover, it has been shown by Watson and Donohue (unpublished) that, of all the possible ways of forming specific sets of pairs of bases, only two sets give symmetrical relationships to the glycosidic bonds. One of these sets satisfies the requirements only very poorly, and a suitable structure cannot be built using it. The other is the specific pairing incorporated in the proposed structure. Thus, even if no analytical data had been available, a sufficiently self-confident model-builder could have arrived at the correct specific pairing. Naturally, the knowledge of the observed base ratios made this step easier to take.

As is well known, the present analytical data give very strong support to this pairing, since, as was first pointed out some time ago by Chargaff,[14] the amount of adenine is found to be practically the same as the amount of thymine, and the amount of guanine the same as that of cytosine, for all sources of DNA so far studied, although the adenine/guanine ratio can vary considerably from one source to another. The most recent analytical evidence[15] shows that the base ratios expected to be 1:1 are indeed very close to this.

The hydrogen-bonding of the bases is also supported by physical-chemical data, but this will not be reviewed here.

It should be clearly realized that the specific pairing of the bases is the direct result of the regular helical nature of the backbone. Without this regularity, many different pairs of bases could be formed. Thus we can now see that the helical diffraction pattern and the analytical data are both reflections of the same thing—namely, the regularity of the phosphate-sugar backbone.

The DNA fibers exist in two forms—the crystalline (A) and the so-called "paracrystalline" (B), depending on the humidity. The structure proposed by Watson and Crick was for the B form, but any proof of the structure must come from A, as this gives much better pictures. The complete interpretation of these extremely beautiful X-ray photos by the King's College workers is eagerly awaited. Until this has been accomplished, no structure can be regarded as completely proved.

It is worth noting that, since the structure is sprinkled with diads (strictly, pseudo-diads) perpendicular to the fiber axis, the phase determination more closely corresponds in difficulty to that for a structure with a center of symmetry. If it were not for this (since a true center of symmetry is impossible because of the asymmetric atoms of the backbone), the correct structure would be much more difficult to establish.

Note that there is, as yet, no *direct* evidence as to whether the helices are right-handed or left-handed (or, less likely, a mixture of both). The proposed model is right-handed. The model-builders were unable to construct a satisfactory left-handed model, but this may merely reflect a lack of ingenuity on their part. Some direct evidence on this point would be most desirable.

Finally, it is highly unlikely that the structure is an "artifact" produced by the extraction process, since similar X-ray patterns have been obtained from intact biological material, such as oriented sperm heads (Wilkins and Randall[9]) and bacteriophage.[5]

* On leave of absence from the Medical Research Council Unit, Cavendish Laboratory, Cambridge, England.

[1] A. R. Todd, these PROCEEDINGS, **40**, 752–759, 1954.

[2] W. T. Astbury and F. O. Bell, *Nature*, **141**, 747, 1938; W. T. Astbury in *Nucleic Acid, Symposia Soc. Exptl. Biol.*, **1**, 66, 1947.

[3] M. H. F. Wilkins, R. G. Gosling, and W. E. Seeds, *Nature*, **167**, 759, 1951.

[4] R. F. Franklin and R. G. Gosling, *Acta Cryst.*, **6**, 673, 1953; *ibid.*, **6**, 678, 1953.

[5] M. H. F. Wilkins, A. R. Stokes, and H. R. Wilson, *Nature*, **171**, 738, 1953.

[6] R. E. Franklin and R. G. Gosling, *Nature*, **171**, 740, 1953.

[7] R. E. Franklin and R. G. Gosling, *ibid.*, **172**, 156, 1953.

[8] M. H. F. Wilkins, W. E. Seeds, A. R. Stokes, and H. R. Wilson, *ibid.*, **172**, 759, 1953

[9] M. H. F. Wilkins and J. T. Randall, *Biochim. et Biophys. Acta*, **10**, 192, 1953.

[10] J. D. Watson and F. H. C. Crick, *Nature*, **171**, 737, 1953.

[11] F. H. C. Crick and J. D. Watson, *Proc. Roy. Soc. London, A*, **223**, 80, 1954.

[12] This in no way conflicts with the recent suggestion of Schachman and Dekker that there are occasional random breaks in the phosphate-sugar backbone. The X-rays see clearly only the idealized, repeating pattern.

[13] W. Cochran, F. H. C. Crick, and V. Vand, *Acta Cryst.*, **5**, 581, 1952.

[14] E. Chargaff, *Experientia*, **6**, 201, 1950.

[15] See, for example, G. R. Wyatt, in V. T. Bowen, *The Chemistry and Physiology of the Nucleus* (New York: Academic Press, 1952), p. 201; E. Chargaff and R. Lipshitz, *J. Am. Chem. Soc.*, **75**, 3658, 1953.

*SOME RELATIONS BETWEEN DNA AND RNA**

By Alexander Rich† and J. D. Watson

GATES AND CRELLIN LABORATORIES OF CHEMISTRY AND KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated by Linus Pauling, May 7, 1954

There are two nucleic acids: DNA (desoxyribonucleic acid) and RNA (ribonucleic acid). Both are found in all complex organisms, whether plant or animal and, together with the proteins, are thought to be among the most important biological substances in the cell. Like the proteins, they are macromolecular and consequently capable of great specificity. DNA is considered to function as a genetic substance and may, in fact, form the specific element of the chromosome.[1] About the functions of RNA, we possess little definite information. It has been implicated in protein synthesis, but only indirectly. The really interesting thing about both the nucleic acids is that we know very little about how they function chemically in a cell.

This uncertainty arises in part from the complex structure of the nucleic acids. Both are polymeric compounds formed from the linear aggregations of the nucleotides, their fundamental subunit. Four main types of nucleotides exist, and they are believed to be randomly situated along the chain. A polynucleotide chain may contain thousands of nucleotides, and thus an almost infinite number of specific nucleic acids is possible. However, despite the indeterminacy of the nucleotide sequence, a certain degree of regularity exists.

In the first place, the union between successive nucleotides is always the same. The main internucleotide linkage in both DNA and RNA is 3′,5′-phosphate diester linkage.[2, 3] Nucleic acids are thus long-chain molecules in which the sugar-phosphate backbone is highly regular, which derive their individuality from the order in which specific purine and pyrimidine bases are attached to the backbone.

The second level of regularity arises from the spatial configuration of the backbone. We learn from X-ray diffraction studies that all DNA's investigated, no matter what their source, have a similar structure.[4] The same is true of all RNA's.[5]

Up to now we have had success in understanding only one of these two structures. DNA appears to be a two-stranded helical structure in which the two polynucleotide chains are joined together by hydrogen bonds between the purine and pyrimidine bases.[4, 6, 7] The hydrogen-bonding arrangement is both regular and specific and always unites the bases together in pairs. Adenine is specifically paired with thymine and guanine with cytosine. The resulting structure is unique, for the specific pairing produces a complementary relationship between the sequence of bases on the two chains. If we know the sequence on one chain, we will automatically have the sequence on the opposing chain. In addition to producing a structure which seems to fit the X-ray data, specific pairing furthermore provides a simple explanation for the analytical observation that, in all DNA's examined, the molar content of adenine is very nearly equal to that of thymine, and that of guanine to that of cytosine.[8]

The most attractive feature of the two-stranded complementary helix is the fact that it suggests an answer to the question of how DNA can replicate itself exactly, a function it must possess if it is a genetic material.[9] The complementary structure

fits this requirement neatly if we make the assumption that one strand can serve as a template for the formation of its complement. We visualize, then, a mechanism involving initial separation of the two strands, with each of the separated strands serving as a template for its complement—the whole process occurring in zipper-like fashion. This method of replication is likely to be very exact, as the necessity for specific pairing is absolute, and misformed pairs will not fit into the structure.

We should also like to know how DNA influences the physiology of the cell. An obvious possibility is that it controls, either directly or indirectly, the synthesis of specific proteins.

Several objections may be cited against a direct role of DNA in protein synthesis. In the first place, we can see no obvious complementary relationship between the shapes of the individual amino acids and the surface of the DNA molecule. Second, protein synthesis appears to proceed normally for some time in cells which lack a nucleus. Both Actabularia[10] and the reticulocyte stage of red blood cells[11] are examples in which protein synthesis occurs in the absence of nuclear DNA. In addition, the sites of greatest protein synthesis as measured by isotope incorporation are the microsomes and the mitochondria, particles in which DNA is completely absent. It would thus appear that protein synthesis often occurs in the absence of DNA.

We consider it plausible to suppose, like many others,[12] a connection between RNA and protein synthesis. Under such a scheme, DNA could control RNA synthesis, with RNA responsible for protein synthesis. Several considerations lead us to favor this hypothesis. First, DNA is lacking entirely in some plant viruses and is replaced by RNA.[13] Second, RNA is found in large amounts in both the microsomes and mitochondria,[14] the particles which are the sites of high protein synthesis.

We shall not be able to check a structural relationship between RNA and protein synthesis or between RNA and DNA until we know the structure of RNA. This compound appears more complex than DNA in several fundamental ways. The chemical formula is not yet established with certainty. While the main internucleotide linkage is similar to DNA (a 3′,5′-phosphate diester linkage), the possibility is still open that the structure is branched.[2] If such branches exist, they most likely arise at the hydroxyl group in the 2-position of a ribose residue in the main chain. Such a branch could not exist in DNA, owing to the absence of this hydroxyl group. Branching of this type has been suggested by many workers,[15, 16] although there is no evidence which is convincing. This problem remains a key one for the chemist to resolve.

The analytical composition of the bases in RNA also seems more complex than that in DNA. The experimental results shown in Table 1 indicate the probable existence of two classes of RNA. The first class is represented by RNA's derived from the plant viruses and appears to indicate a fairly random distribution of the various bases. No evidence for the existence of the specific pairs of bases is provided. On the contrary, in RNA's of the second type the molar amount of adenine is approximately equal to that of uracil (for pairing purposes, uracil can be considered equivalent to thymine), and the amount of guanine tends to equal that of cytosine. This second class includes many RNA's from sources other than plant

viruses. A few exceptions to the 1:1 ratio are found, but it is not certain that they are significant. It is obvious that in many RNA's the specific pairs are found, and this must be significant. One possibility is the existence of two types of RNA structure, in only one of which are the base pairs utilized. This, however, cannot be true, since, as mentioned earlier, RNA of all sources produces the same X-ray pattern. A simple interpretation of the analytical data does not appear possible.

Until recently there have existed no satisfactory X-ray diffraction data on RNA. We are therefore beginning to obtain X-ray data from various sources of RNA. We have found that it is possible to draw fibers from RNA preparations and to obtain X-ray diffraction patterns from them.[5]

These photographs do not show the high degree of fibrous orientation which can be obtained from DNA. We do not know whether this is owing to partial degradation of our specimens or whether it is because the molecule is intrinsically less

TABLE 1

PURINE AND PYRIMIDINE COMPOSITION OF VARIOUS RNA'S

Source	Adenine*	Uracil	Guanine	Cytosine	Analysis by†
Rabbit liver:					
Mitochondria	0.193	0.199	0.308	0.302	Crosbie, *et al.* (1953)[17]
Microsomes	0.195	0.199	0.305	0.300	
Cell sap	0.193	0.197	0.298	0.296	
Whole cytoplasm	0.192	0.213	0.304	0.292	
Calf brain	0.216	0.206	0.318	0.260	Deluca, *et al.* (1953)[18]
Calf thymus	0.162	0.157	0.352	0.330	Cohn and Volkin (1953)[15]
Carp nucleotropomyosin	0.166	0.178	0.348	0.318	Hamoir (1952)[19]
Sea urchin oöcytes	0.200	0.220	0.307	0.298	Vincent (1952)[20]
Carcinoma of liver (human)	0.098	0.071	0.407	0.426	Chargaff, *et al.* (1950)[21]
Fowl carcinoma	0.140	0.120	0.460	0.280	Harris, *et al.* (1950)[22]
Tobacco mosaic virus	0.296	0.264	0.251	0.186	Knight (1952)[23]
Cucumber mosaic virus	0.258	0.301	0.255	0.187	Knight (1952)[23]
Potato virus X	0.342	0.212	0.215	0.235	Knight and Dorner (1953)[24]
Tobacco necrosis virus	0.278	0.257	0.245	0.225	Markham (1953)[25]
Tomato bushy stunt	0.248	0.252	0.280	0.219	Markham and Smith (1951)[26]
Southern bean mosaic	0.258	0.252	0.260	0.232	Knight and Dorner (1953)[24]
Turnip yellow virus	0.227	0.222	0.172	0.382	Markham and Smith (1951)[26]

* All ratios have been adjusted to total 100 per cent.
† Superior reference numbers refer to notes at end of article.

regular and may not pack well. One of our RNA fiber photographs is illustrated in Figure 1. For comparative purposes, Figure 2 shows X-ray photographs of the crystalline and paracrystalline forms of DNA. Despite the lack of resolution in the RNA photograph, several points of interest emerge: (1) The pattern is dominated by strong reflections on or near the meridian at 3.3 and at 4.0 A. This most likely results from a stacking of purine and pyrimidine bases approximately on top of each other, arranged roughly perpendicular to the fiber axis. This interpretation is supported by the negative birefringence of the fibers.[5] A similar arrangement is found in the DNA structure.[4, 7] (2) The repeat distance along the fiber axis is 25–28 A. This can be seen in some photographs, where the 5.3 A equatorial reflection shows a layer-line periodicity of intensity in a direction parallel to the fiber axis. There is also a subrepeat of half this distance, as is indicated by the strong near-meridional reflections on the second layer line. This feature is also present in the DNA photographs. (3) There is a noticeable absence of any meridional or near-meridional reflection between approximately 13 and 4 A.

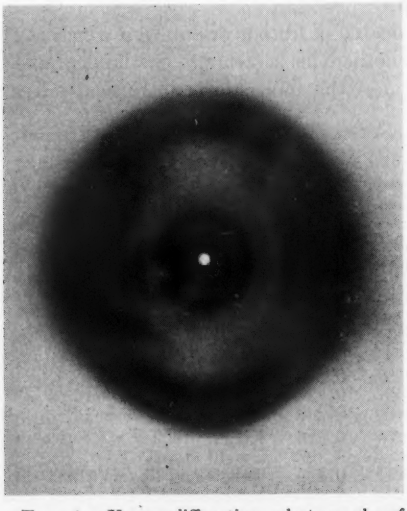


FIG. 1.—X-ray diffraction photograph of RNA fiber. Experimental conditions: fiber axis vertical; Cu $K\alpha$ radiation; 10-cm. cylindrical camera filled with helium gas; relative humidity, 66 per cent.

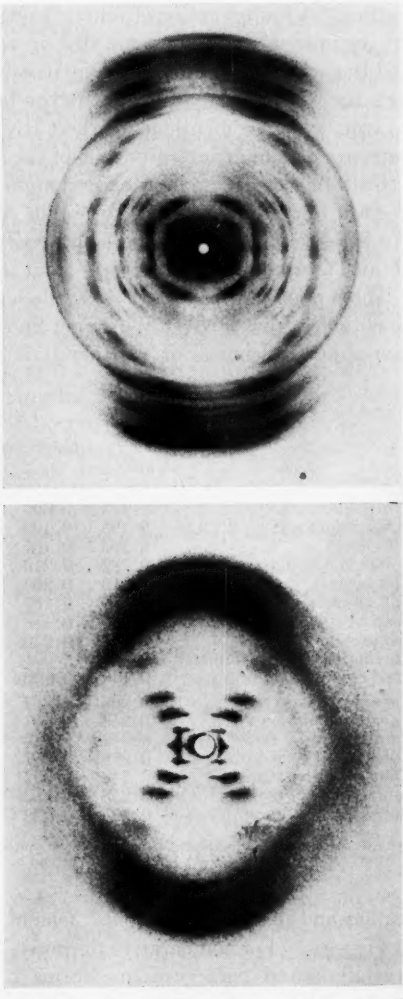


FIG. 2.—X-ray diffraction photographs of DNA fibers. *a*, Crystalline state of DNA (from M. H. F. Wilkins, W. E. Seeds, A. R. Stokes, and H. R. Wilson, *Nature*, **172,** 759–762, 1953). *b*, Paracrystalline state of DNA (from R. E. Franklin and R. G. Gosling *Nature*, **171,** 740–742, 1953).

This is again similar to the DNA photographs, where it is interpreted in terms of a helical structure. (4) The equatorial reflections are not well resolved but seem to be compatible with a fiber of diameter approximately 21–25 A. (5) The exact details of the patterns are strongly influenced by the water content of the specimen.[5] The innermost reflection at 22 A (probably equatorial) disappears at very low

relative humidities but appears strongly when the humidity is high. The exact distance of this spacing varies somewhat with water content. However, the general appearance of the photograph remains approximately the same; hence the structure cannot have undergone any serious modifications while taking up water.

The X-ray pattern therefore suggests a DNA-like structure for RNA. However, since the DNA model is based upon complementary base ratios which are not found in many RNA's, this suggestion has many difficulties. It is possible that non-complementary side chains may arise from a complementary main structure, but proof of this awaits more direct chemical evidence of branches in RNA.

We have been able to construct single-chain helical models for RNA in which the free ribose hydroxyl group is satisfactorily hydrogen-bonded to a negatively charged phosphate group. However, we have not been able to form satisfactory intramolecular hydrogen bonds between the bases, which, in this model, remain free to form external hydrogen bonds. Experimentally, it appears essential to determine whether the bases in RNA form significant numbers of intramolecular hydrogen bonds, as in DNA. A re-examination of the electrometric titration curve on undegraded specimens may settle this point.

In the above, we have discussed RNA structure from the viewpoint of its possible connection with protein synthesis. We might also briefly consider the possibility of a genetic role for RNA. This arises from the complete absence of DNA in the plant viruses and its replacement by RNA. In these viruses the genetic material must be the RNA component or the protein component, or possibly both. We might hope to obtain a clue by examining the base ratios. It is conceivable that the RNA's which may have a genetic role would show complementarity. The results, however, seem just the opposite. Plant virus RNA's show great departure from the 1:1 ratio, while RNA's from sources to which we need not necessarily postulate a genetic role (e.g., microsomes, mitochondria) often provide beautiful examples of complementarity. We have no explanation for this finding.

Summary.—We have discussed possible functions and relations between DNA and RNA. Current structural studies on RNA have been described. Further chemical and crystallographic work is necessary before we can discover the relationship between the structure of RNA and the origin of protein specificity.

Acknowledgment.—We have profited from discussions with Dr. L. E. Orgel.

* Contribution No. 1917 from the Gates and Crellin Laboratories of Chemistry. This investigation was supported in part by a grant from the National Foundation for Infantile Paralysis.

† United States Public Health Service.

[1] O. T. Avery, C. M. MacLeod, and M. McCarty, *J. Exptl. Med.*, **79**, 137–157, 1944; A. D. Hershey and M. Chase, *J. Gen. Physiol.*, **36**, 39–56, 1952.

[2] D. M. Brown and A. R. Todd, *J. Chem. Soc.*, pp. 52–58, 1952.

[3] D. M. Brown, G. D. Fassman, D. I. Magrath, A. R. Todd, W. Cochran, and M. M. Soolfson, *Nature*, **172**, 1184–1185, 1953.

[4] M. H. F. Wilkins, A. E. Stokes, and H. R. Wilson, *Nature*, **171**, 738–740, 1953.

[5] A. Rich and J. D. Watson, *Nature* **173**, 995–996, 1954.

[6] J. D. Watson and F. H. C. Crick, *Nature*, **171**, 737–738, 1953.

[7] R. E. Franklin and R. G. Gosling, *Nature*, **171**, 740–742, 1953.

[8] E. Chargaff, *Experientia*, **6**, 201–209, 1950; G. R. Wyatt, *J. Gen. Physiol.*, **36**, 201–205, 1952.

[9] J. D. Watson and F. H. C. Crick, *Nature*, **171**, 964–967, 1953.

[10] J. Brachet and H. Chantrenne, *Nature*, **168**, 950, 1951.

[11] H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley, and P. H. Lowy, *J. Biol. Chem.*, **196**, 669–694, 1952.
[12] T. Caspersson, *Symposia Soc. Exptl. Biol.*, **1**, 127–151, 1947; J. Brachet, *Symposia Soc. Exptl. Biol.*, **1**, 207–224, 1947; A. L. Bounce, *Nature*, **172**, 541, 1953.
[13] R. Markham, *Advances in Virus Research*, **1**, 315–332, 1953.
[14] A. Claude, *Science*, **97**, 451–458, 1943.
[15] W. E. Cohn and E. Volkin, *J. Biol. Chem.*, **203**, 319–332, 1953.
[16] D. O. Jordan, *Progr. Biophys. and Biophys. Chem.*, **2**, 51–89, 1951.
[17] G. W. Crosbie, R. M. S. Smellie, and J. N. Davidson, *Biochem. J.*, **54**, 287–292, 1953.
[18] H. A. Deluca, R. J. Rossita, and K. P. Strickland, *Biochem. J.*, **55**, 193–201, 1953.
[19] G. Hamoir, *Biochem. J.*, **50**, 140–144, 1952.
[20] W. C. Vincent, these PROCEEDINGS, **38**, 139–145, 1952.
[21] E. Chargaff, B. Magasanik, E. Vischer, C. Green, R. Doniger, and D. Elson, *J. Biol. Chem.*, **186**, 51–67, 1950.
[22] R. J. C. Harris, R. N. Beale, and E. M. F. Roe, *J. Chem. Soc.*, pp. 1397–1407, 1952.
[23] C. A. Knight, *J. Biol. Chem.*, **197**, 241–249, 1952.
[24] C. A. Knight and R. W. Dorner, *J. Biol. Chem.*, **205**, 959–967, 1953.
[25] R. Markham, in P. Fildes and W. E. Van Heyningen, *The Nature of Virus Multiplication* (Cambridge: At the University Press, 1953, pp. 85–95).
[26] R. Markham and J. D. Smith, *Biochem. J.*, **49**, 401–406, 1951.

BIOSYNTHESIS OF NUCLEIC ACID IN ESCHERICHIA COLI

BY ELLIS BOLTON

DEPARTMENT OF TERRESTRIAL MAGNETISM, CARNEGIE INSTITUTION OF WASHINGTON

Communicated by M. A. Tuve, May 26, 1954

The nucleic acids of different organisms are similarly constructed and are apparently endowed with common functions. An underlying unity appears also to characterize their biological synthesis. Biochemical studies have shown that microörganisms,[1] birds,[2] and mammals[3] utilize carbon dioxide for the synthesis of nucleic acid purines and pyrimidines; that a number of features of pentose synthesis are common to bacteria[4] and yeast;[5] and that nucleotide-synthesizing enzymes occur in many kinds of cells.[6] However, knowledge of nucleic acid synthesis is not complete for any species. It is not known what kinds of subunits enter nucleic acid macromolecules or what kinds of processes link the units together into strands. Investigations which couple the techniques of microbiology with tracer isotopes and chromatography promise to supply answers to these questions.

This paper reports the results of experimental studies which have surveyed nucleic acid biosynthesis in growing *Escherichia coli*. The results reveal some characteristics of the materials and processes which contribute to the formation of nucleic acid macromolecules.

Escherichia coli is a heterotrophic bacterium which grows readily in simple, chemically defined culture media. At 37° C. its generation time is about one hour. While growing exponentially, it will utilize relatively simple carbon sources such as glucose and carbon dioxide to supply its carbon requirements. It will also utilize a host of supplemental carbon compounds, and it ordinarily makes use of each in a specific fashion. Nutritional characteristics such as these can be ex-

ploited by means of radioactive tracer techniques and chromatographic analysis to reveal much of the nucleic acid biochemistry of the cell.

A typical experimental procedure followed for this purpose is as follows: About 5 mg. (wet) cells are grown aerobically for an hour in a medium containing glucose, salts, and an appropriate tracer-labeled compound. The crop of cells (10 mg. wet) is harvested and washed in the centrifuge. The resulting pellet is fractionated with cold trichloroacetic acid, (TCA) alcohol and ether, and hot trichloroacetic acid. The hot-TCA fraction is then analyzed by paper chromatography, spectrophotometry, and isotope determinations. Figure 1 shows a radioautograph of a

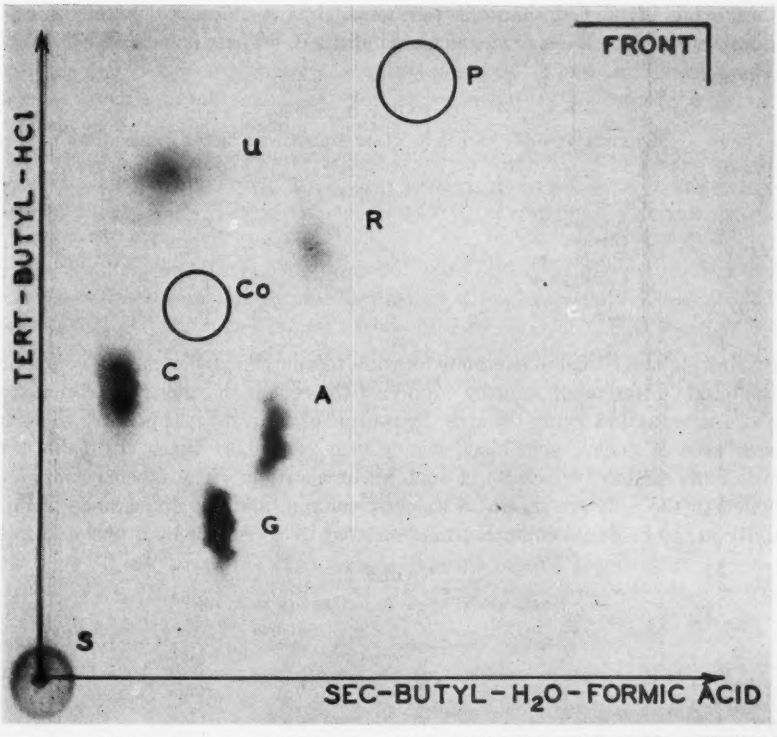


FIG. 1.—Radioautograph of chromatogram of a C^{14}-labeled nucleic acid hydrolyzate. *A*, adenine; *G*, guanine; *C*, cytidylic acid; *U*, uridylic acid; *R*, ribose; *Co*, desoxycytidylic acid; *P*, orthophosphoric acid.

two-dimensional chromatogram of the components which are in the hot-TCA fraction of cells grown in the presence of uniformly C^{14}-labeled glucose. Each of these components may be removed from the chromatograms and estimated by chemical and isotopic determinations. About 300 μgm. of nucleic acid are usually analyzed in this way. Desoxyribonucleic acid is, quantitatively, a minor component making up only about one-sixth of the total. For this reason it cannot easily be studied by the routine procedure described. Therefore, the data of this paper apply principally to the bulk component, ribonucleic acid.

In Table 1 are summarized some results of a quantitative analysis of the nucleic acid derived from cells grown in the presence of $C^{14}O_2$. The data show that both the purines and both the pyrimidine nucleotides are labeled and that the isotope is equally distributed between the members of a pair. No C^{14} is found in the pentose. This sugar is derived from the original purine nucleotide segment of the macromolecule. When the pyrimidine nucleotides and cytidylic and uridylic acids are also chemically degraded to yield the free base, all the C^{14} originally in the nucleotide is found in the pyrimidine ring. Therefore, this experiment demonstrates that the CO_2 supply of the culture medium serves as a source of carbon for the purine and pyrimidine rings in *E. coli*. It is also evident that the pyrimidines are more highly labeled than the purines. Thus a difference in the modes of synthesis of the two classes of chemicals is indicated. These facts have been known for some years.[7]

TABLE 1

UTILIZATION OF $C^{14}O_2$ FOR NUCLEIC ACID SYNTHESIS BY *E. coli*

	Specific Radioactivity (C/Sec/μM)
Purines:	
Adenine	110
Guanine	110
Pyrimidine nucleotides:	
Cytidylic acid	180
Uridylic acid	180
Ribose	0

By shifting the C^{14}-label from one location to another in the carbon compounds in the basal culture medium or by supplying the cells with other labeled materials, one at a time, and carrying through the routine of analysis, it is possible to explore a large area of nucleic acid biochemistry in a very short time. In Table 2 are shown some qualitative results of such an exploration. The labeled compounds supplied to the cells are shown in the first column, and the appearance of radioactivity in the bacterial component is indicated by a cross or by a plus and minus

TABLE 2

SOURCES OF NUCLEIC ACID CARBON IN *E. coli*

	Adenine	Guanine	Cytidylic Acid	Uridylic Acid	Ribose
C^{14}-glucose	×	×	×	×	×
2-C^{14}-glucose	×	×	×	×	×
1-C^{14}-glucose	×	×	×	×	..
$C^{14}O_2$	×	×	×	×	..
$HC^{14}OOH$	×	×	..	..	..
$C^{14}H_2O$	×	×	..	..	..
C^{14}-glycine	×	×	..	..	..
C^{14}-serine	×	×	±	±	..
C^{14}-threonine	×	×	±	±	..
C^{14}-aspartate	±	±	×	×	..
C^{14}-glutamate	±	±	×	×	..
$C^{14}H_3COOH$	±	±	×	×	..

NOTE: All cultures grown in glucose-salts medium with indicated radioactive additive.

where only traces of C^{14} appear. The labeled compounds which the cell has utilized fall into three main classes. In the first class are grouped those compounds which contribute to all four of the bacterial ribonucleotides. 1-C^{14}-glucose and radioactive carbon dioxide do not label ribose. In the second class

are listed the compounds which label the purines especially, and in the third are those which label the pyrimidines especially. Some other C^{14}-labeled materials have also been studied and are discussed below.

These data indicate that carbon atoms 2–6 of glucose contribute to bacterial ribose. Carbon atom 1 contributes poorly, if at all. The long series of biochemical events taking place between glucose and ribose have been intensively studied.[4, 5]

The source of the carbon atoms of the purines was one of the first topics in nucleic acid synthesis to be studied with carbon isotopes.[2] CO_2, formate, and glycine were shown to contribute to the purine ring. Similar observations have now been made for a number of organisms. In *E. coli* it has been shown that both threonine and serine can give rise to glycine, but glycine does not give rise to serine or threonine.[8] Hence glycine is in a key position. Thus, in *E. coli* as in other organisms, CO_2, formate, and glycine take part in the formation of the purine ring.

In the case of the pyrimidines, CO_2, aspartate, glutamate, and acetate contribute carbon. Aspartate here appears to be the compound of central importance, since, by means of the Krebs cycle[9] it becomes labeled from CO_2, acetate, and glutamate. The pathway leading from the precursors to pyrimidines has been studied in *E. coli* by Weed and Wilson.[10]

The results shown in Table 2 point to the relatively simple materials which can serve as sources of carbon for nucleic acid synthesis in *E. coli*. They also indicate the over-all pattern of the flow of carbon into nucleic acid. Studies such as these not only are fundamental to an understanding of the complex of integrated activities in the bacterial cell but can be put to specific use in order to reveal the nature of the more complex chemicals which take part in the construction of polynucleotide strands.

In Table 3 are given the results of some experiments in which the incorporation of radioactive carbon dioxide was again studied but in which glucose-salts media

TABLE 3

UTILIZATION OF $C^{14}O_2$ IN THE PRESENCE OF C^{12}-COMPETITORS

CULTURE	SUPPLEMENT	RELATIVE SPECIFIC RADIOACTIVITY OF BACTERIAL COMPONENT (PER CENT OF CONTROL PURINE)			
		Adenine	Guanine	Cytidylic Acid	Uridylic Acid
1	None (control)	100	100	170	170
2	Adenine	<5	<5	170	170
3	Guanine	100	<5	170	170
4	Cytosine	100	100	30	30
5	Uracil	100	100	20	20

were supplemented with nitrogenous bases. The results are given in terms of specific radioactivity, which has been expressed as per cent of the value found for the purines in culture 1, the control culture. As observed above (Table 1), radioactive carbon dioxide labels the purines and the pyrimidine compounds. When the cultures containing supplements are examined, it is evident that marked suppressions in the incorporation of C^{14} occur. These suppressions are specifically associated with the kind of supplement used. Thus in culture 2, to which adenine was added, radioactive carbon dioxide supplies a very small amount of tracer, and hence of carbon, to the nucleic acid purines. The nonradioactive carbon which now supplants the CO_2 carbon in the purines of culture 2 was, of course, supplied

by adenine. Thus the adenine derived from the culture medium is preferentially utilized by the cell to make nucleic acid. Exogenous adenine supplies carbon to each of the nucleic acid purines; thus we may conclude that adenine can be converted to guanine.[11] Pyrimidine labeling remains unaffected as a result of adenine supplementation. In culture 3, where exogenous guanine was supplied, only polynucleotide guanine could be shown to derive carbon from the exogenous purine. Thus guanine is not readily, at least, converted to adenine. In cultures 4 and 5 exogenous cytosine and uracil supplied carbon to nucleic acid pyrimidines and were quite freely interconverted. It is evident from these data that external sources of purines and pyrimidines can serve as precursors to nucleic acid. They do so effectively and specifically, acting as preferred sources of carbon.

In Table 4 is shown another series of results from isotopic competition experiments[12] in which nucleosides and nucleotides have been studied. In the case of the "adenine series" it is evident from the suppressions of C^{14}-utilization that the free base and the riboside are effective carbon sources but that the nucleotides are relatively poor sources. The fact that the purine nucleotides are utilized to some degree indicates very strongly that impermeability of the cell wall may not be invoked to account for their behavior. That they are not so readily utilized as the base or the riboside demonstrates that they are not readily converted to these substances.

TABLE 4

UTILIZATION OF $C^{14}O_2$ IN THE PRESENCE OF C^{12}-COMPETITORS

		Relative Specific Radioactivity of Bacterial Component (Per Cent of Control Purine)			
Culture	Supplement	Adenine	Guanine	Cytidylic Acid	Uridylic Acid
1	None (control)	100	100	170	170
2	Adenine	<5	<5	170	170
3	Adenosine	<5	<5	170	170
4	Adenosine-5-phosphate	50	50	170	170
5	Yeast adenylic acid (a + b isomers)	50	50	170	170
6	Uracil	100	100	20	20
7	Uridine	100	100	10	10
8	Uridylic acid	100	100	20	20

In the case of the "uracil series" the exogenous materials are about equally effectively utilized to supply the pyrimidine requirements of nucleic acid. Results similar to these are obtained when corresponding "guanine" and "cytosine" series are also studied with radioactive carbon dioxide. In the guanine case, however, there is little conversion to adenine.

These competition experiments with radioactive carbon dioxide show that carbon of the several supplements is preferentially utilized in nucleic acid synthesis. But they do not reveal the mechanisms by which the supplement is utilized. It is not learned from them whether the ring structure of a base or the ribosidic linkage in the nucleoside or the phosphate ester linkage in the nucleotide is preserved during the utilization of the supplement. However, each of these problems can be solved by appropriate isotope experiments.

For example, if C^{14}-glucose, uniformly labeled, is supplied to growing cells, and it is the only source of carbon available, then all the carbon locations in the nucleic acid newly made by the cell become labeled. If a similar culture is prepared, but

with nonradioactive adenine as a competitor, it is found that polynucleotide purine derives over 90 per cent of its carbon from the exogenous source. Thus every carbon location of the bacterial purine ring derives some, and very likely all, of its carbon from the exogenous purine. Similar results follow for the corresponding cases of guanine, cytosine, and uracil supplementation. It is reasonable to conclude, therefore, that all of the ring structure of the externally supplied nitrogenous base is utilized during the formation of nucleic acid.

In order to determine whether the nucleosides and nucleotides are utilized as such to form nucleic acid, some experiments with C^{14}- and P^{32}-labeled pyrimidine nucleotides were carried out. In these experiments uniformly C^{14}-labeled cytidylic and uridylic acids were supplied to separate cultures of growing *E. coli*, as were the corresponding P^{32}-labeled compounds. After an hour's growth the cells of each culture were harvested and analyzed as described. In addition, the bacterial pyrimidine nucleotides were degraded to yield free cytosine and uracil, and each of these residues was analyzed. The results are shown in Table 5.

TABLE 5

UTILIZATION OF LABELED PYRIMIDINE NUCLEOTIDES FOR NUCLEIC ACID SYNTHESIS

LABELED COMPOUND	SP. ACT. (C/SEC/μM)	SPECIFIC RADIOACTIVITY OF BACTERIAL COMPONENT					
		Cytidylic Acid	Cytosine*	Ribose†	Uridylic Acid	Uracil*	Ribose†
C^{14}-cytidylic acid	160	113	56.5	56.5	90	49	41
P^{32}-cytidylic acid	94	~1	..	..	~1	..	..
C^{14}-uridylic acid	117	60	33	27	70	36.5	33.5
P^{32}-uridylic acid	169	~1	..	..	~1	..	..

* From 6 *N* HCl hydrolyzate.
† Estimated by difference.

Thus, in the cultures supplied C^{14}-cytidylic acid, appreciable C^{14} was incorporated into the bacterial pyrimidine nucleotides, but in the parallel phosphorus-labeled culture practically no radiophosphorus had been taken up. A similar result was observed for the "uridylic acid" pair of the cultures. It has been shown by separate measurements that the cells are permeable to each of the four labeled compounds. It is clear, therefore, that the exogenous pyrimidine nucleotides are incorporated into nucleic acids in a manner which dissociates the phosphorus atom from the carbon atoms. This result has been confirmed by isotopic competition experiments with P^{32}-labeled inorganic phosphate and nonradioactive pyrimidine nucleotides. It was again observed that the exogenous nucleotides did not supply phosphorus for nucleic acid synthesis. Thus externally supplied pyrimidine mononucleotides are utilized to supply carbon, but they are not utilized as intact units for ribonucleic acid synthesis in growing *E. coli*.

When the distributions of C^{14} in the bacterial components were determined (second, third, fifth, and sixth columns of Table 5), it was observed in each case that the pyrimidine ring and the ribose moiety were approximately equally labeled. These findings imply that pyrimidine nucleosides can enter as units into the structure of polynucleotide strands. They also indicate that pyrimidine interconversions can occur at the nucleoside level.[13]

These results have been confirmed by a series of competition experiments in which the utilization of C^{14}-cytidylic acid in the presence of nonradioactive cytosine and sugars was studied. Some of the results are given in Table 6. These results

show that no suppression in the utilization of C^{14}-cytidylic acid occurred as a result of the presence of cytosine or of the sugar compounds. In addition, C^{14}-ribose is not utilized by these cells. Thus the exogenous nucleotide was utilized in preference to the base or sugar compounds. Since the phosphorus of the pyrimidine nucleotide does not accompany its carbon (Table 5), it is concluded that pyrimidine nucleosides can act as intermediates in nucleic acid synthesis, entering as units into the structure of bacterial polynucleotides.

TABLE 6

SPECIFIC UTILIZATION OF C^{14}-CYTIDYLIC ACID FOR NUCLEIC ACID SYNTHESIS

CULTURE	SUPPLEMENT*	SPECIFIC RADIOACTIVITY OF BACTERIAL COMPOUND (C/SEC/μM)†	
		Cytidylic Acid	Uridylic Acid
1	None (control)	33.0	25
2	Cytosine, ribose	31.7	25
3	Cytosine, ribose-5-phosphate	32.5	25

* 1 μM C^{14}-cytidylic acid; 2 μM each supplement per culture.
† Adenine and guanine are very weakly labeled (sp. act. <1 c/sec/μM).

The problem of purine nucleotide utilization was examined by means of the isotopic competition method, using either P^{32}-labeled inorganic phosphate or uniformly C^{14}-labeled glucose as the tracer compounds. Neither adenosine-5-PO_4 nor yeast adenylic acid influenced the utilization of labeled inorganic phosphate for the synthesis of nucleic acid. Guanylic acid, inorganic pyrophosphate, and adenosine triphosphate behaved in a similar fashion. However, when the various adenylic and guanylic acids were tested with C^{14}-glucose, some utilization of their carbon could be detected. It appeared, therefore, that exogenous purine nucleotides, like pyrimidine nucleotides, did not enter as units into nucleic acid.

Consequently, purine nucleosides were examined by the isotopic competition method, using C^{14}-glucose as the tracer material. The purine nucleosides were found to be effective competitors, suppressing the uptake of C^{14} into bacterial nucleic acid. However, while they contributed to the purine base, they did not appear to contribute to the ribose moiety of nucleic acid purine ribotide. Therefore, a search was made for possible sources of the ribose that was attached to the purine ring in bacterial nucleic acid.

TABLE 7

UTILIZATION OF C^{14}-GLUCOSE FOR NUCLEIC ACID SYNTHESIS

CULTURE	SUPPLEMENT	SPECIFIC RADIOACTIVITY OF BACTERIAL COMPONENT (C/SEC/μM)							
		Adenylic Acid*				Guanylic Acid*			
		a	b	Adenine†	Ribose‡	a	b	Guanine†	Ribose‡
1	None (control)	37	35	20	16	34	..	20	14
2	Adenosine	17	17	2	15	17	15	5	11
3	Cytidine	23	25	17	7	18	19	13	5.5
4	Uridine	29	28	20	8.5	23	25	20	4

* Specific activity of isomers a and b, respectively.
† After 1 *N* HCl hydrolysis.
‡ Estimated by difference.

Preliminary experiments indicated that pyrimidine nucleosides and nucleotides at moderately high levels (1–2 μM/ml) might serve as sources of purine ribose. This suggestion was critically examined by isotopic competition experiments with C^{14}-glucose and pyrimidine-containing materials. In Table 7 are given some results of experiments which have used adenosine, cytidine, and uridine as com-

petitors in separate cultures containing C^{14}-glucose. In these experiments the routine analytical procedure was modified in order to yield the bacterial purine nucleotides. These compounds were then purified by chromatography[14] and analyzed. Table 7 shows that the purine nucleotides of the unsupplemented culture contain the largest amounts of radioactivity. The purine nucleotides of the supplemented cultures contain smaller amounts of C^{14}. Clearly, carbon from each of the various supplements is utilized for purine nucleotide synthesis. Examination of the second and third columns of each section in Table 7 reveals that the purine and pyrimidine nucleoside supplements are utilized differently. Adenosine contributes largely to the adenine and guanine portions of the nucleotides, while cytidine and uridine contribute largely to the sugar moiety. Cytidine appears also to affect guanine synthesis. Thus the ribose portion of the exogenous pyrimidine nucleoside becomes a part of the purine nucleotide segment of nucleic acid. Under the conditions of this experiment well over half the purine-associated ribose arose in this way. The details of the mechanisms by which this comes about are not yet known, although in over-all appearance the result is equivalent to what the enzyme chemist recognizes as "transglycosidation."[15] In the example shown in Table 7 a unit containing a pyrimidine nucleoside would serve as the "ribosyl" donor, and a purine-containing unit, synthesized by the cell from glucose, would serve as the acceptor.

Conclusion.—By means of isotopic tracer methods which take advantage of the nutritional characteristics of growing bacteria, it is possible to determine which substances serve as sources of nucleic acid carbon. It is also possible to insert suspected precursors at various places along the synthetic streams which form nucleic acid and to decide what parts they play while contributing to the flow of carbon.

Thus studies using media supplemented by pyrimidine nucleosides specify some of the processes involved in the synthesis of nucleic acid. In these cases the purines are manufactured from glucose-carbon, while the ribose associated with them is derived from the externally supplied pyrimidine nucleoside. This transfer of ribose involves a process much like, and perhaps identical with, transglycosidation. Meanwhile, the pyrimidine nucleosides are also incorporated into nucleic acid as units. The same mechanisms operate when pyrimidine nucleotides are studied. Here, however, the phosphorus of the nucleotide is replaced by phosphorus derived from the inorganic phosphorus of the medium. Although the exact nature of the mechanism which inserts phosphorus into nucleic acid has not been revealed, we would suggest that phosphorus compounds, perhaps of the uridine-triphosphate type, react with nucleosides to form the nucleic acid linkages in ribonucleic acid chains.

[1] A. L. Koch, F. W. Putnam, and E. A. Evans, Jr., *J. Biol. Chem.*, **197**, 105, 1952.
[2] J. M. Buchanan, J. C. Sonne, and A. M. Delluva, *J. Biol. Chem.*, **173**, 81, 1948.
[3] M. R. Heinrich and D. W. Wilson, *J. Biol. Chem.*, **186**, 447, 1950.
[4] S. S. Cohen, *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 221, 1953.
[5] B. L. Horecker, *J. Cellular Comp. Physiol.*, Suppl. 1, **41**, 137, 1953.
[6] H. M. Kalckar, *Biochim. et biophys. acta*, **12**, 250, 1953.
[7] E. T. Bolton, P. H. Abelson, and E. Aldous, *J. Biol. Chem.*, **198**, 179, 1952.
[8] P. H. Abelson, E. T. Bolton, R. Britten, D. B. Cowie, and R. B. Roberts, these PROCEEDINGS, **39**, 1020, 1953.

[9] R. B. Roberts, D. B. Cowie, R. Britten, E. Bolton, and P. H. Abelson, these PROCEEDINGS, **39**, 1013, 1953.

[10] L. L. Weed and D. W. Wilson, *J. Biol. Chem.*, **189**, 435, 1951.

[11] Cf. Koch, Putnam, and Evans, *op. cit.*

[12] D. B. Cowie, E. T. Bolton, and M. K. Sands, *J. Bacteriol.*, **62**, 63, 1951. Cf. Abelson, Bolton, Britten, Cowie, and Roberts, *op. cit.*

[13] R. B. Merrifield and D. W. Woolley, in W. D. McElroy and B. Glass (eds.), *Phosphorus Metabolism* (Baltimore: Johns Hopkins Press, 1952), II, p. 354.

[14] R. Markham and J. D. Smith, *Biochem. J.*, **52**, 552, 1952.

[15] H. M. Kalckar, in W. D. McElroy and B. Glass (eds.), *The Mechanism of Enzyme Action* (Baltimore: Johns Hopkins Press, 1954), p. 675.

INFORMATION TO CONTRIBUTORS

THE PROCEEDINGS is the official organ of the NATIONAL ACADEMY OF SCIENCES and of the NATIONAL RESEARCH COUNCIL for the publication of brief accounts of important current researches of members of the ACADEMY and of the COUNCIL and of other American investigators. The PROCEEDINGS will aim especially to secure prompt publication of original announcements of discoveries and wide circulation of the results of American research among investigators in other countries and in all branches of science.

ARTICLES should be brief. The viewpoint should be comprehensive in giving the relation of the paper to previous publications of the author or of others and in exhibiting, where practicable, the significance of the work for other branches of science. Elaborate technical details of the work and long tables of data should be avoided, but authors should be precise in making clear the new results and should give some record of the methods and data upon which they are based.

MANUSCRIPTS should be prepared with a current number of the PROCEEDINGS as a model in matters of form, and should be typewritten in duplicate with double spacing, the author retaining one copy. Illustrations should be confined to text figures of simple character, though more elaborate illustration may be allowed in special instances to authors willing to pay for their preparation and insertion. Particular attention should be given to arranging tabular matter in a simple and concise manner.

MANUSCRIPTS will be accepted only from members of the Academy or from chairmen of the divisions of the National Research Council who will assume responsibility for the propriety and scientific standards of the paper and should be addressed to *Proceedings of the* NATIONAL ACADEMY OF SCIENCES, Attention Miss Mary D. Alexander, University of Chicago Press, 5750 Ellis Avenue, Chicago 37, Illinois. Articles of which a member of the Academy is author or co-author may extend to 6 printed pages, those by non-members to 4 printed pages; space above these limits will be billed to the author at $21.00 per page. Accompanying the manuscript should be a statement by the author or his representative that he assumes responsibility for the charges if incurred.

GALLEY PROOF will be sent, with the understanding that charges for author's corrections shall be billed to him. Author should therefore make final revisions on the typewritten manuscripts. Page proofs will not be sent.

REPRINTS should be ordered when proof is returned, on the reprint order form which will be inclosed with the proof. The order blank gives a tabular estimation of the cost of reprints; a copy will be supplied at any time upon request.

CONTENTS

Page

BIOCHEMISTRY.—THE EFFECT OF DIABETES AND FASTING ON LIVER GLUCOSE-6-PHOSPHATASE . . . . . *By James Ashmore, A. Baird Hastings, and Frances B. Nesbett* 673

BIOCHEMISTRY.—COLLAGEN STRUCTURES CONSIDERED AS STATES OF AGGREGATION OF A KINETIC UNIT. THE TROPOCOLLAGEN PARTICLE . . . . . *By Jerome Gross, John H. Highberger, and Francis O. Schmitt* 679

BIOCHEMISTRY.—ON THE GLUTAMATE-PROLINE-ORNITHINE INTERRELATION IN *Neurospora Crassa* . . . . . *By Henry J. Vogel and David M. Bonner* 688

GENETICS.—CAROTENOID DIFFERENCES IN *Lycopersicon:* HYBRIDS OF AN UNUSUAL RACE OF *L. Pimpinellifolium* . . . . . *By G. Mackinney, C. M. Rick, and J. A. Jenkins* 695

MATHEMATICS.—LIMIT THEOREMS FOR HOMOGENEOUS STOCHASTIC PROCESSES . . . . . *By S. Bochner* 699

MATHEMATICS.—SUR LES GROUPES D'EILENBERG-MAC LANE. II. . . . *By Henri Cartan* 704

MATHEMATICS.—A NONLINEAR DIFFERENTIAL-DIFFERENCE EQUATION OF GROWTH . . . . . *By W. J. Cunningham* 708

MATHEMATICS.—A NEW CLASS OF SIMPLE LIE ALGEBRAS . . . *By Marguerite Straus Frank* 713

MATHEMATICS.—GENERAL EXISTENCE AND UNIQUENESS PROOF FOR SPATIALLY HOMOGENEOUS SOLUTIONS OF THE MAXWELL-BOLTZMANN EQUATION IN THE CASE OF MAXWELLIAN MOLECULES. . . . . *By Dietrich Morgenstern* 719

MATHEMATICS.—ON THE ANALYSES OF $\{m\} \otimes \{1^k\}$ AND $\{m\} \otimes \{k\}$ . . . . . *By Francis D. Murnaghan* 721

MATHEMATICS.—THE REDUCTION OF THE INNER PRODUCT OF TWO IRREDUCIBLE REPRESENTATIONS OF S_n . . . . . *By G. de B. Robinson and O. E. Taulbee* 723

MATHEMATICS.—ON A CLASS OF SEMISIMPLE RESTRICTED LIE ALGEBRAS . . . . . *By George B. Seligman* 726

MATHEMATICS.—HAMARTIEXÉRESIS AS APPLIED TO TABLES INVOLVING LOGARITHMS. . . . . *By Horace S. Uhler* 728

MATHEMATICS.—EXAMINATION OF METHODS OF ATTACK ON THE SECOND CASE OF FERMAT'S LAST THEOREM . . . . . *By H. S. Vandiver* 732

PATHOLOGY.—THE AGGLUTINATING AND SENSITIZING CAPACITY OF ANTISERA TO SHEEP RED CELLS AFTER VARYING DEGREES OF PHOTO-OXIDATION . . . . . *By Albert Tyler, M. Lorraine Fiset, and Robin R. A. Coombs* 736

PHYSICS.—SOME PHYSICAL ASPECTS OF THE STABILITY OF PARALLEL FLOWS . *By C. C. Lin* 741

NATIONAL ACADEMY OF SCIENCES.—SYMPOSIUM ON THE STRUCTURE AND FUNCTION OF NUCLEIC ACIDS . . . . . *By Linus Pauling* 747

CHEMISTRY.—CHEMICAL STRUCTURE OF THE NUCLEIC ACIDS . . . *By Alexander R. Todd* 748

CHEMISTRY.—THE COMPLEMENTARY STRUCTURE OF DNA . . . . . *By F. H. C. Crick* 756

CHEMISTRY.—SOME RELATIONS BETWEEN DNA AND RNA . . . . . *By Alexander Rich and J. D. Watson* 759

BIOCHEMISTRY.—BIOSYNTHESIS OF NUCLEIC ACID IN *Escherichia Coli*. . . . *By Ellis Bolton* 764

Page
673
679
688
695
799
704
708
713
719
721
723
726
728
732
736
741
747
748
756
759
764